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# The effect of Visfatin and Adiponectin on metabolic parameters of an animal model of type 2 diabetes

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**THURAYA SAEED ALI ALSHAMISI**
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A Thesis
Submitted to
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In Partial Fulfillment of the Requirements
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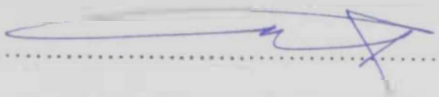

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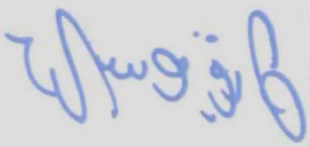

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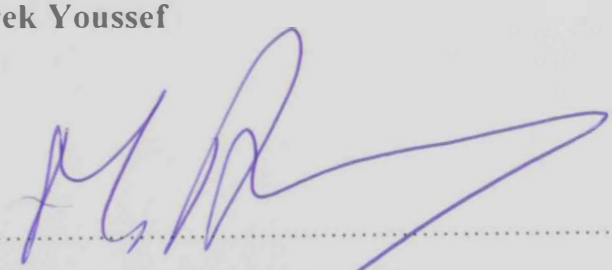

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# DECLARATION

The work presented in this thesis has not been accepted in substance for any other degree and is not concurrently being submitted in candidature for any other degree

Signed.....Thuo.....

Candidate

Date.....10/10/20.....

This is to certify that the work here submitted was carried out by the candidate. Due acknowledgment has been made for any assistance received

Signed.....

Supervisor

Signed.....

Supervisor

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# ABSTRACT

# ABSTRACT

**Background:** Diabetes mellitus (DM) is one of the most common exocrine disorders affecting more than 6 % of the world's population. The incidence and prevalence of this chronic disease are on the rise in many parts of the world including the Middle East. DM is caused by defective insulin production, release and/or function. The causes and nature of abnormal insulin metabolism is less than clear. While defective insulin secretion is observed in type 1 diabetes, insulin resistance is predominant in type 2 diabetes, which affects more than 94% of the diabetic patients.

**Materials and Methods:** Rats between the age of two and three months were divided in to nine groups each consisting of 12 rats: Goto Kakizaki (GK) normal, GK treated with visfatin, GK treated with adiponectin, Wistar diabetic rats treated with visfatin, Wistar diabetic rats treated with adiponectin, Normal Wistar treated with visfatin, Normal Wistar rats treated with adiponectin, untreated diabetic Wistar rats, and normal untreated Wistar rats.

Diabetes in Wistar rats was induced by a single intraperitoneal injection of streptozotocin (60 mg $kg^{-1}$). One week from induction of diabetes, selected groups received intraperitoneal injection of (10 ng/kg body weight) either adiponectin or visfatin for 14 weeks. All animals from all groups were sacrificed after 14 weeks of treatment for blood plasma biochemistry analysis. The pancreas was rapidly removed and representative fragments were taken to be used for immunohistochemical, immunofluorescence, and electron microscopy studies.

**Results:** Adiponectin and visfatin improved glucose tolerance in Wistar as well as GK rats. In addition, the number and quality of pancreatic beta cells improved after treatment with adiponectin and visfatin leading to increase in the plasma level of insulin. Liver and kidney parameters including lactic acid dehydrogenase, alkaline phosphatase, blood urea nitrogen decreased significantly after treatment with either adiponectin or visfatin.

**Conclusion:** Adiponectin and visfatin ameliorate several metabolic parameters in animal models of type 1 and type 2 diabetes, resulting in improved glycemic control.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| BMI | Body Mass Index |
| WHO | World Health Organization |
| VLDL | Very-Low-Density Lipoprotein |
| PP | Pancreatic Polypeptide |
| DM | Diabetes Mellitus |
| Stz | Streptozotocin |
| GK | Goto Kakizaki |
| OGTT | Oral Glucose Tolerance Test |
| PBS | Phosphate Buffer Saline |
| BUN | Blood Urea Nitrogen |
| CRE | Creatinine |
| TP | Total Protein |
| IDDM | Inslin dependent diabetic mellitus |
| NIDDM | Non-Inslin dependent diabetic mellitus |
| PPEF1 | pre –Bcell colony enhancing factor 1 |
| NAD+ | Nicotinamide adenine dinucleotide |
| PBS | Phosphate buffered saline |
| DPX | Di-N-Butyle Phthalate in Xylene |
| PCR | Polymerase chain reaction |
| RNA | Ribonucleic acid |

| | |
|---|---|
| PHOS | Phosphorus |
| DNA | Deoxyribonucleic acid |
| RQ | Relative Quantification |
| SD | Standard deveiation |
| Hmox1 | Heme oxygenase (decycling) 1 |
| Hspa4 | Heat shock 70 kDa protein 4 |
| Gcg | Glucagone gene |
| Pdx1 | Pancreatic and duodenal homeobox 1 |
| Gpx2 | Glutathione peroxidase 2 |
| Insr | Insulin receptor |
| ELIZA | Enzyme-linked immunosorbent assay |

# LITERATURE REVIEW

# I. Diabetes

## 1.1. Definition

Diabetes mellitus (DM) is a disease defined by WHO as a metabolic disorder of multiple etiology, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include short and long-term damage, dysfunction and failure of various organs (1).

The effects of the disease may be acute or chronic, involving many organs, including the eye, the kidney, peripheral nerves and large arteries. Primary diabetes mellitus is traditionally divided into either type 1 (insulin dependent, IDDM) or type 2 (non-insulin dependent, NIDDM). The classification is important because of the different genetic backgrounds, clinical presentations, metabolic effects, treatment and consequences of the two types. Diabetes may also be secondary to other disorders (2).

## 1.2. Classification

Usually, diabetes was classified according to the patient's age at onset of symptoms. In 1979, the NIH diabetes data group proposed a plan to divide diabetes into two main types (3):

1. Insulin dependent and,
2. Non – insulin dependent

But this "therapeutic classification" proved unacceptable as more information on pathogenesis and etiology of diabetes mellitus accumulate in 1997.

The International Committee of Diabetologists suggested that several changes in the classification of diabetes that has been endorsed by the American Diabetes Association and the World Health Organization can include the following (3):

1. The terms - insulin – independent diabetes mellitus and Non – insulin – dependent diabetes mellitus as their acronyms IDDM and NIDDM to be eliminated.
2. The term type 1 diabetes and type 2 diabetes are retained with Arabic rather than roman numerals.

### 1.2. a. Type 1 diabetes mellitus

Type 1 is associated with ketosis in the untreated state. Approximately 9 % of diabetics from North America and 20 % of diabetics from the Scandinavian countries have type 1 diabetes (3). It is most common in young individuals but may occur in non-obese adults. It is a metabolic disorder in which circulating insulin is nearly absent. When plasma glucose is elevated the pancreatic B cells fails to react and hyperglycemia develops. There are three main target tissues of insulin (liver, muscle and fat). In the absence of insulin, glucose uptake by these tissues is impaired. In addition, alterations in fat metabolism lead to the production and accumulation of ketone bodies (3,4).

Type 1 diabetes is believed to be a result of an exposure of a genetically predisposed person to infection or toxin or environmental insult resulting in the development of antibodies that destroy pancreatic B cells. Infectious and environmental factors implicated in the etiology of type 1 diabetes include viruses (mumps, rubella, coxsackievirus, B4), toxic chemical agents such as vacor (a nitrophenylurea rat poison) and other destructive cytotoxins such as hydrogen cyanide from spoiled tapioca or cassava root (3).

### 1.2. b. Type 2 diabetes mellitus

Type 2 diabetes, formerly classified as non-insulin-dependent diabetes (NIDDM) occurs in individuals with a relative, rather than absolute insulin deficiency and may be accompanied by insulin resistance. It accounts for more than 90 % of diabetes cases in the United States. The patients are usually adults, ages range to 40 years old and above, with some degree of obesity.

They do not normally require insulin to survive, although their insulin secretory capacity tends to depreciate, and many needs insulin treatment to achieve optimal glucose control. Ketosis rarely occurs, but if present, it is usually an outcome of associated stress, trauma or infection. Although the etiology of the primary defect in type 2 diabetes is unclear and presumably multi factorial, it is well known that tissue insensitivity to insulin has been noted in most type 2 patients regardless of weight. Subgroups of type 2 diabetes have recently been identified:

1-Obese type 2 diabetes:

Patients with this type of diabetes mostly present with insulin resistance. Several investigators consider that this type of diabetes may represent only one facet of a metabolic syndrome. They noted that the well established association of hyperglycemia, hyperinsulinemia, dyslipidemia and hypertension, leads to coronary artery disease and stroke. It may result from a genetic defect producing insulin resistance, especially when obesity aggravates the degree of insulin resistance (3).

The prevalence of obesity is more than 30% of Chinese and Japanese patients with type 2 diabetes. It is present in 60 – 70 % of North America while Europeans or Africans with type 2 diabetes approach 100 % of type 2 patients. Among Pima Indians or Pacific Islanders from Nauru or Samoa, patients with type 2 diabetes have insensitivity to endogenous insulin, which is correlated with the presence of mainly abdominal distribution of fat, producing an abnormal high waist to hip ratio (3).

2-Non obese type 2 diabetes

Poor insulin release by the pancreatic B cell seems to be the main defect in non-obese type 2 diabetic patients, but some insulin resistance at the post receptor level can also be detected. However, the degree of insulin resistance does not appear to be clinically applicable to the treatment of most non-obese type 2 patients, who generally respond to appropriate therapeutic supplements of insulin in the absence of rare associated conditions such as lipoatrophy of acanthuses nigricans (3). At present, type 2 diabetes is considered to be of an idiopathic origin. On the other hand, with developments in biotechnology, a variety of etiologic genetic abnormalities has been documented within a heterogeneous group, mainly those presenting with clinical and laboratory manifestations similar to those in the non-obese type 2 subgroup(3).

When the genetic defect has been defined, these patients were reclassified within this group as "other specific types"(3).

**1.2. c. Gestational diabetes**

Gestational diabetes is defined as carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy. It occurs in 2% to 5% of all pregnancies (5). Although the risks associated with gestational diabetes are well recognized, it remains uncertain whether screening and treatment to reduce maternal glucose levels reduce these risks.

## 1.3. Symptoms and diagnosis

Recent studies indicate that early detection of diabetes symptoms and treatment can decrease the chance of developing the complications of diabetes. The symptom of type 1 and type 2 diabetes vary in and depends on the severity and type of the disease. Diabetes is associated with frequent urination, unusual thirst, extreme hunger, unusual weight loss and extreme fatigue and irritability. Long standing diabetic may have some or all of the following symptoms depending on the degree of morbidity: blurred vision, cuts/bruises that are slow to heal, tingling/numbness in the hands/feet and recurring skin, gum, or bladder infections (4).

## 1.4. Complication of diabetes mellitus

Complications of DM are mainly either acute or chronic:

### 1.4. a. Acute complications of DM:

The most important acute complications of DM are metabolic: diabetic ketoacidosis, hypoglycaemia, lactic acidosis and non-ketotic hyperosmolar coma. Other acute complications include acute infections and acute neuropathy (6). Acute infections may be the presenting complaint in type 2 DM. They may include: candidal infections, carbuncles, boils and other staphylococcal skin infections, osteomyelitis, urinary infections, pneumonia, tuberculosis and other systemic bacterial infections in the diagnosed diabetic, finger pulp infections caused by non-sterile finger pricks (6). Acute motor or sensory neuropathy may be seen in various guises during or after a period of poor metabolic control. These neuropathies commonly present in the form of amyotrophic pain and skin tenderness with weakness and wasting of the upper thigh muscles. These complications may resolve with improved glycemic control (often with insulin) (6).

### 1.4. b. Chronic complications of DM

Most of the chronic complications of diabetes result from disease of either the large blood vessels (macroangiopathy) or the small blood vessels (microangiopathy). Macroangiopathy is responsible for a high prevalence of coronary, peripheral and cerebral artery disease in diabetics. Hypertension coexists with DM in about 50% of patients. Accelerated atherosclerosis occurs at a young age and runs an aggressive course in DM, especially in women. It accounts for most deaths, particularly in type 2 DM. Microangiopathy is a generalized microvascular (capillary) disorder that is specific to DM and clinically most apparent in the eyes, kidneys and nerves (6).

## 1.5. Treatment and management of diabetes mellitus

Treatment of all types of DM is intended to lower blood glucose level at most possible normal average by administering insulin or oral hypoglycemic drugs and/or dietary modification. In type 1 DM, patients are given insulin and go on dietary therapy. This allows the body to use up glucose as an energy source. In type 2 DM, dietary modification is the main factor of treatment. In addition, life style modification is also recommended. But physical effort should be recommended by the patient's treating doctor. The patient is usually recommend to practice light exercise such as walking and swimming to use up a part the body's glucose (7).

Patient and family education, diet and nutritional recommendations, physical exercise, treatment of obesity, oral anti-hyperglycemic agents, insulin and management of associated conditions and complications forms the cornerstone of the management of DM and vary from country to country (5).

## 1.6. Epidemiology of diabetes mellitus in the UAE

The prevalence of impaired glucose tolerance (IGT) and diabetes based on the World Health Organization and the UAE Ministry of Health (5) between the years 1998-2000 on 6,609 male and female (2,363 UAE nationals and 4,246 expatriates) individuals above the age of 21 years were as follows:

Total DM 19.6%, IGT 15.2%
UAE nationals DM 24%, IGT 17.9%
Expatriates DM 17.4%, IGT 13.4%

In the UAE, prevention of type 2 DM epidemic in adolescents is encouraged by reducing childhood obesity, which remains a major public health challenge. The emergence of type 2 DM in children is closely linked with obesity. Recent study shows that the presence of type 2 DM in the UAE pediatric population has a major public health implication (8). The high prevalence of type 2 DM in the UAE adult population and obesity in children (8) pose a sever health risk in the population.

A more recent result on the epidemiology of diabetes reported increases in the prevalence of DM (34). Diabetes takes its financial toll on the UAE. According to the Ministry of Health, heart disease related to diabetes was the first killer in the UAE last year, comprising 31 per cent. Moreover, the UAE spends between US$100 and US$200 million (Dh367 to Dh734 million) annually on treating diabetes (9). Research and statistics report on diabetes across the UAE suggest that the disease will cost an estimated Dh10 billion by the year 2020 if the condition is not treated (10).

# II. Obesity

## II.1. Definition and causes

Body weight is most commonly assessed by body mass index (BMI) which is calculated by dividing an individual's weight measured in kilograms by their height in meters squared. Therefore overweight is defined as a BMI of 25.0-29.9 kg/m2 and obese is a BMI ≥30.0 kg/m2 (38, 39), which is about a gain of 20% or more of the ideal weight.

The causes of obesity are many and multi factorial. Factors contribution to the development of obesity may be influence by one or more of the following:

1- The imbalance of intake daily calories: Obesity is the result of the increased intake of daily calories compared to the energy need of the individual. The net increase in calorie will be deposited in the body as fat (11, 12).

2- Culture: People are usually served large amounts of foods that are most readily available instead of choosing foods that are most nutritious. That's why Today's culture promotes eating habits that leads to obesity (12).

3- Gender: Muscle uses more energy than fat. Men have more muscle than women, and burn 10 percent to 20 percent more calories than women do at rest. For this reason, women are more likely to be obese (6).

4- Age: The amount of muscle in human body tends to decrease as we age, and fat will accounts for a greater percentage of weight. This lower muscle mass leads to a decrease in metabolism and lower glucose uptake. In addition metabolism also slows with ageing. Taken together, these changes reduce calorie needs. If food intake is not adjusted, human body will gain weight (11).

5- Hormonal imbalance: Hormonal and metabolic diseases such as Cushing Syndrome may contribute to weight gain and obesity (14, 15).

6- Genes play a part in how human body balances calorie and energy. As of October 2005, 176 human obesity cases due to single-gene mutation in 11 different genes have been reported (16). Genes can make persons more susceptible to gaining weight, by influencing the amount of body fat and fat distribution. Children whose parents are obese also tend to be overweight. Genes can also play role in laziness, lethargy, and lack of movement (13).

7- Emotional or Psychological Factors: Food is often a source of solace or celebration. If we celebrate we may go out to a big dinner. If a friend is grieving, we bake them a pie. The relationship between obesity and psychological status is very strong and vary from person to person, some people eat greedily in the case of joy, and others eat very large amounts when they face a specific problem, while others abstain from food completely and get the loss of many of the weight when exposed to emotional problem (17).

## II.2. Complications of obesity

According to the 2002 WHO World Health Report, overweight and obesity lead to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance. Risks of coronary heart disease, ischaemic stroke and type 2 diabetes mellitus increase steadily with increasing BMI. Raised BMI also increases the risk of cancer of the breast, colon, prostate, endometrium, kidney and gallbladder (13).

There is a nine-year reduction in life expectancy amongst obese patients, the risk being markedly amplified if they also smoke(13). Generalized obesity (fat distributed around the whole body) results in alterations in blood circulation and heart function, while central/abdominal obesity (fatness mainly around the chest and abdomen) further restricts chest movements and alters breathing function. Fat around the abdomen is also a major contributor to the development of diabetes, hypertension, and alterations in blood lipid (triglycerides and cholesterol) concentrations (13).

## II.3. Obesity and diabetes mellitus

Mortality is significantly higher among subjects with greater than average body weight compared to those with less than average body weight. In fact many studies have reported association between diabetes and obesity (18). Although an inherited liability to develop diabetes is probably the most important factor in the etiology of type 2 diabetes. Obesity and diabetes are separate and distinct risk factors for ischemic heart disease and their combined presence in the obese diabetic person may be particularly lethal. Most diabetic patients die from ischemic heart disease. It is uncertain whether the risk of death from ischemic heart disease can be reduced by treatment with either insulin or oral hypoglycemic agents. The risk of death from ischemic heart disease can, however, certainly is diminished by reducing body weight (18).

## II.5. Epidemiology of Obesity in the UAE

Obesity is currently an escalating epidemic in many countries in the world including the Arabian Gulf region. The United Arab Emirates enjoys a high income per capita, which is considered among the highest in the world (19) after oil production, which began in the 1960s. Tremendous growth in population and urbanization also accompanied changes in the pattern of life. The country has undergone significant changes in nutritional and life style habits.

Such changes are expected to have an impact on the magnitude of chronic diseases including obesity. 71% of married women and 56% of married men were found to be obese (19). However, the problems of obesity and related diseases in the United Arab Emirates are not limited to citizens who make up less than 20% of the population. The country's development depends heavily on expatriate workers, mostly from Bangladesh, India, Islamic Republic of Iran, Pakistan, the Philippines, and other Arab countries. Obesity was common in all groups. Approximately three-quarters of all were either obese (BMI $\geq$ 30) or overweight (BMI 25–29) (20).

The World Health Organization reported that the prevalence of obesity has reached alarming numbers globally. It was estimated that in 2005, 1.6 billion adults, worldwide, are overweight, including 400 million obese. A study published in year 2000 reported that more than 50% of men and women in the UAE are either overweight or obese (20). These rates are also increasing in other countries in the region.

The Department of Nutrition and Health at the UAE University in Al Ain reported that about a quarter of children between the ages of 8 and 12 years old are overweight (8). The prevalence of obesity is increasing rapidly in the United Arab Emirates (UAE) especially among females. A recent study showed that the prevalence of obesity was associated positively with age (young female) and negatively with the degree of education (21). Thus prevention programs should begin in childhood, since 21% of women are already obese by the third decade (21). In addition, The Centre for Arab Genomic Studies of the United Arab Emirates announced that the rate of obesity among UAE nationals amounted to nearly 70% of the total citizens, which is indicator of risk (22, 23). Therefore, only prompt action will prevent the preeminent burden to the health services in the near future (20).

# III. Pancreas

## III.1. Histology

The pancreas located on the posterior body wall, deep to the peritoneum, and consists of four regions: uncinate process head, body, and tail. It is about 25 cm long, 5 cm wide, and 1 to 2 cm thick, and it weighs approximately 150 g. Its flimsy connective tissue capsule forms septa which subdivide the gland into lobules. The vascular and nerve supply of the pancreas, as well as its system of ducts travels in these connective tissue compartments. The pancreas produces exocrine and endocrine secretions. The endocrine component of the pancreas, islets of Langerhans are scattered among the exocrine secretory acini (24).

## III.2. Endocrine Pancreas

The pancreas consists of two functionally different organs:

1. The Exocrine pancreas - the major digestive gland of the body. The major role of the products of the exocrine pancreas (the digestive enzymes) is to process ingested foodstuffs for absorption (25).

2. The Endocrine pancreas -is the source of insulin, glucagon, somatostatin, and pancreatic polypeptide.The hormones of the endocrine pancreas modulate the aspect of cellular nutrition from rate of absorption to cellular storage or metabolism of nutrients. The dysfunction of the endocrine pancreas or abnormal responses to its hormones by target tissues may result in serious disturbances in nutrient homeostasis, including the important clinical syndromes grouped under the name of diabetes mellitus (3). The endocrine pancreas consists of 0.7 to 1 million islands of small endocrine glands. The islets of Langerhans scattered within the glandular substance of the exocrine pancreas.

The islet level comprise of 1-1.5% of the total mass of the pancreas and weighs about 1-2g in adult humans (3).

## III.3. Insulin

Insulin is a protein with 51 amino acids, contained within two di-sulfide chains: The molecular weight of human insulin is 5808 Da. Endogenous insulin has a circulatory half life of 3-5 minutes. It is catabolised mainly by insulinases in liver, kidney and placenta. Approximately 50% of insulin is removed in a single pass through the liver.

The human pancreas secretes around 40-50 units of insulin per day in normal adults. Plasma glucose levels below 80-100 mg/dl (4.4-5.6 mmol/L) do not stimulate insulin release. It has been confirmed that the presence of glucose is required (in-vitro systems) for most other known regulators of insulin secretion to be efficient in reaction to exogenous stimuli (3). Glucose is the most effective stimulant of insulin release. Glucose is known to enter the pancreatic B cell by passive diffusion, which is facilitated by a specific membrane protein called glucose transporter-2. By good quality of its relatively low similarity for glucose, this protein is more effective in facilitating the transport of glucose during postprandial hyperglycemia than at the lower levels of blood glucose during an overnight fast (3).

Insulin action begins when insulin binds with the receptor on the surface of target cell membrane. Many cells of the body appear to have specific cell surface insulin receptors. In fact, liver and muscle cells, insulin binds with these receptors to trigger biological response to the hormone .The first major organ reached by insulin via the bloodstream is liver. In the liver, insulin increases both protein and triglyceride synthesis and VLDL formation. It also inhibits gluconeogenesis and promotes glycolysis through its effects on enzymes of the glycolytic pathway (3). In addition, insulin inhibits catabolism and acts to reverse the catabolic events of the post absorptive state by inhibiting hepatic glycogenolysis and cytogenesis.

The second organ reached by insulin is muscle. Insulin promotes protein synthesis in muscle by increasing the amino acid transport and stimulating ribosomal protein synthesis. In addition, insulin promotes glycogen synthesis and restores glycogen stores, expended during muscle activity. Therefore by increasing glucose transport into the Glycogen synthesis is enhanced by inhibiting the activity of glycogen phosphorylase. On the other hand insulin acts to elevate triglyceride storage in adipocytes by a number of mechanisms

## III.4. Glucagon

Pancreatic glucagon is a single chain polypeptide, which consists of 29 amino acids with a molecular weight of 3485 Da. It is synthesized in the cells of the islets of Langerhans and derived from a 160-amino-acid precursor molecule, which is five to six times bigger than glucagon. Glucagon secretion is inhibited by glucose – in contrast to the effect of glucose on insulin secretion.

Glucagon is a humoral factor for the production of energy available to the tissues between meals. When ingested food is not available for absorption, glucagon stimulates the breakdown of stored glycogen. It maintains the hepatic output of glucose. Compared to insulin it promotes energy storage in a variety of tissues. The liver represents the major target organ for glucagon because of its geographic closeness to the pancreas with portal vein glucagon concentrations reaching as high as 300-500 pg/ ml (100-166 pmol/L)(3,26).

## III.5. Somatostatin

The gene for somatostatin is located at the long arm of chromosome 3. It codes for 116-amino-acid peptide, pre-pro-somatostatin from which carboxyl terminal is cleaved with somatostatin, a 14-amino acid cyclic polypeptide with a molecular weight of 1640 Da. It was first identified in the hypothalamus and gets its name because of its ability to inhibit release of growth hormone (pituitary somatotropin).

Since that time, somatostatin has been identified in a number of tissues, including many areas of the brain, in the gastrointestinal tract, pancreas, central nervous system and the pancreas . Somatostatin acts in several ways to restrain the movement of nutrients from the intestinal tract into the circulation. It prolongs gastric emptying time, decreases gastric acid and gastrin production. It diminishes pancreatic exocrine secretion; decreases splanchnic blood flow, and delay xylose absorption .

## III.6. Pancreatic Polypeptide

Pancreatic polypeptide (PP) is a 36 amino-acid peptide with a molecular weight of 4200 Da. We know little about its biosynthesis. The circulating levels of the peptide increases in response to a mixed meal. In healthy subjects, basal levels of PP can average up to 24 ±4 pmol/L and it may increase due to a range of factors including old age, alcohol abuse, diarrhea, chronic renal failure, hypoglycemia and other inflammatory disorders. Values above 300 pmol/ L are found in most patients with pancreatic endocrine tumor such as glucagonoma or vipoma and in all patients with tumors of the pancreatic F cell. Mainly 20 % of patients with insulinoma and one – third of those with gastrinomas also have PP plasma concentrations of greater than 300 pmol/L (3).

# IV. Adipose tissue

## IV.1. Endocrine adipose tissue

Adipose tissue insulates and cushions the body and store free fatty acids after food intake. This fatty acid is released when the body in the fasting state. This old definition of adipose tissue role was replaced by new discovery of its endocrine role (27). In early 1987, adipose tissue was identified as a major site of production for adipsin, an endocrine factor that is markedly down-regulated in rodent obesity (28). The characterization of leptin, in adipose tissue, in 1994 established the adipose tissue as an endocrine organ (28). It is a well accepted fact that adipose tissue plays a role in the hormonal regulation of body homeostasis (29). The term adipokines is used to describe hormones released from adipose tissue such as leptin, adiponectin, resistin and visfatin (30).

Adipose tissue produces bioactive peptides (adipokines) involved in metabolism of glucose, lipid and in the regulation of inflammation, coagulation and blood pressure (27). However, the main regulator of adipocyte fat content is insulin (27). Adipose tissue expresses numerous receptors that allow it to respond to signals from traditional hormone system, which explain the current view of its endocrine role of sending out and responding to signals that modulate insulin sensitivity and immunity (31, 28).

The association between accumulation of visceral adipose tissue and insulin resistance is well established in the etiology of obesity and diabetic mellitus (32).

## IV.2. Visfatin

Ten years ago a protein secreted by lymphocyte was identified and called pre-B cell colony enhancing factor1 (PPEF1) (33). PPEF1 has recently been renamed visfatin and shown to be released by adipocytes and increased in visceral adipose tissue compared with subcutaneous adipose tissue (33, 35).

Visfatin was first identified in 2005 as novel member of adipocytokine family (37) with a variety of actions, including the following:

a- Acting as biomarker of acute lung injury.

b- Influence infected fetal membranes.

c- Inhibiting neutrophilic apoptosis in experimental inflammation and clinical sepsis (36).

d- Participating in the maturation of vascular smooth muscle cells through a NAD+ dependent mechanism (37).

e- Participate in the etiopathology of metabolic syndrome (38, 39).

It was reported that the concentration of visfatin correlated positively with fat mass in human (34), supporting previous reports that demonstrated a correlation between plasma visfatin concentration and obesity (33). However, the relationship between obesity and elevated plasma visfatin levels has not been confirmed (35). Moreover, recent studies showed that visfatin level may be related to visceral obesity in man and may account for variation of glucose in individuals (40). A study performed on Chinese individuals comparing metabolic parameters to visfatin concentration found that visfatin concentration is higher in individuals with type 2 diabetes mellitus compared to healthy control subjects (34).

With every year, more and more studies report that in various insulin sensitive tissues such as liver, muscle and fat, visfatin exerted insulin mimetic effects (33-34,41), because it binds and activate the insulin receptor in diabetic type 2 (42). Independently, visfatin is associated with waist-hip ratio and body mass index (BMI) (43), insulin resistance (44), hyperandrogenism in lean patients (44), and dysfunction in early diabetic nephropathy (45). A recent study performed in 2008 concluded that visfatin was significantly lower in the gestational diabetes mellitus pregnant women than pregnant women with normal glucose tolerance (46).

## IV.3. Adiponectin

Adiponectin is a 244 amino acid hormone that is produced in adipose tissue (visceral, subcutaneous and bone marrow fat depots (47-48, 50). Various names such ACRP30, APM1, GBP28, ADIPOQ have been used to describe adiponectin (47). The recent discovery of two adiponectin receptor isoforms has given more interest into the differential effects of various adiponectin isoforms. Adiponectin acts as an insulin sensitizer by enhancing fatty acid oxidation in skeletal muscle and suppressing hepatic glucose production in various animal models (52). Moreover, it was found that adiponectin is lower in males than females (51).

The finding that the concentration of adiponectin is higher in persons with type 1 diabetes than in non-diabetic persons and type 2 diabetes (51, 20) opened the door to study the detailed effect of this peptide starting with epidemiological data present in 2006. It was shown that low level of adiponectin at baseline is associated with increased risk to developing type 2 diabetes (53).

In January 2009 another study demonstrated for the first time that adiponectin levels in subjects with type 2 diabetic nephropathy are strongly positively associated with fasting insulin levels and insulin resistance (54) and high adiponectin level is associated with significantly reduced risk of DM development .The level of adiponectin inversely correlated with body mass index and body fat distribution (55). In a study of obese child and its relation of adiponectin, body fat, gender and puberty, it was demonstrated that increase in adponectin concentration is associated with a significant weight loss and improvement of insulin resistance (57). In vivo and in vitro studies have shown that adiponectin level is inversely related to oral glucose tolerance, insulin sensitivity and cardiovascular problems (47, 56).

Currently, adiponectin has been suggested as a new drug in the treatment of atherosclerosis and metabolic syndrome such as visceral obesity, and insulin resistance in type 2 diabetes mellitus (49).

# OBJECTIVES

# OBJECTIVES

Visfatin and adiponectin, secreted by adipose tissues, are signaling molecules implicated in the development of obesity and consequently type 2 diabetes (64). The aim of this study is to examine the effect of visfatin and adiponectin on the metabolic parameters (weight, glucose, oral glucose tolerance test) of Goto Kakizaki (GK) rats, an animal model of type 2 diabetes.

The specific objectives are as follows:

1. Immunolocalize visfatin and adiponectin in the cytoplasmic organelles of adipocytes and pancreas of GK and Wistar rats.
2. Investigate the short- and long-term effect of visfatin and adiponectin on the pattern of distribution of insulin, glucagon, somatostatin and pancreatic polypeptide in GK and Wistar rats.
3. Determine whether intraperitoneal injection of visfatin and adiponectin will lead to a further enhancement of insulin resistance and worsening of the metabolic parameters in GK and Wistar rats.
4. Examine the relationship between visfatin and adiponectin, obesity and type of diabetes.

# MATERIALS AND METHODS

# I. Materials

## I.1. Experimental animal

Goto Kakizaki (GK) and Wistar male rats (WR) were breaded in the Animal House Facility in the Faculty of Medicine & Health Sciences, UAE University. All rats were housed in a temperature (25°C) and humidity controlled rooms and 12 hours light and dark periods. The animals were fed on standard laboratory animal chew with food and tap water ad libitum .

The rats within age of two and three month, were divided in to nine groups each consisting of 12 rats: 1-GK normal, 2-GK treated with visfatin, 3-GK treated with adiponectin, 4-WR diabetic treated with visfatin, 5-WR diabetic treated with adiponectin, 6- WR normal treated with visfatin, 7- WR normal treated with adiponectin, 8-WR diabetic untreated and 9-WR normal untreated. Diabetes in WR was induced by a single intraperitoneal injection of streptozotocin (60 mg $kg^{-1}$) prepared in 5 mM citrate buffer pH 4.50 (59). The animals were considered diabetic if the random blood glucose levels were equal to or more than 250 mg/dl. One week from induction of diabetes, 3 groups received intraperitoneal injection of (10 ng/kg body weight) adiponectin. In addition, another 3 groups were treated with intraperitoneal injection of 10 ng/kg body weight of visfatin). All animals from all groups were sacrificed after 14 weeks of treatment for blood plasma biochemistry analysis. The pancreas was rapidly removed and representative fragments were taken to be used for immunohistochemical, immunofluorescence and electronmicroscopy studies .

## I.2. Experimental chemicals

Standard laboratory chemicals used for processing of tissues and other laboratory techniques were acquired from either BDH or Sigma. All other kits were acquired from Phoenix (visfatin (141-168) (Human), adiponectin recombinant (rat), insulin and glucagon EIA, mouse-monoclonal anti-adiponectin, antiserum), or Dako (guinea-pig anti insulin, rabbit anti-human glucagon, rabbit anti-human somatostatin, or Jacksons Laboratory (anti rabbit RRX and anti

guinea-pig FITC). Beckman Coulter reagents and Real-time PCR kits, TaqMan® Gene Expression Assay (Applied Biosystems, USA)

### I.3. Experimental equipment

All equipments for immunohistochemistry and electron microscopy were available in the Faculty of Medicine & Health Sciences while biochemical Analysis performed at Clinical Chemistry Department at Al Ain Hospital.

## II. Methods

### II.1. In vivo studies

#### II.1.a. Weight measurement

The weights of normal and visfatin and adiponectin-treated rats were taken weekly and recorded. The average weight for each group was calculated.

#### II.1.b. Blood glucose

Blood glucose levels measured from the tail vein of normal and treated GK and Wistar rats at weekly intervals using a Life Scan Glucometer (Milpitas, CA, USA) to examine whether visfatin and adiponectin has any effect on fasting blood glucose values when compared to untreated rats. All rats were fasted overnight (12 hours) for the measurement of blood glucose.

#### II.1.c. Glucose tolerance test

At the end of the experiments (14 weeks), control and treated rats were fasted overnight (12 hours) prior to the test. Each rat was given an oral glucose load, 2g/kg body weight (59). Blood samples were collected from the tail vein at time 0 (prior to the glucose load), 30, 60 and 120 minutes after the glucose load.

### II.1.d. Tissue collection and tissue processing

At the end of the experiment, normal and treated rats were sacrificed for blood plasma biochemistry analysis and collecting of pancreas and fat tissue. A long abdominal incision was made and the pancreas and epididymal fat tissues were quickly removed. While subcutaneous fat tissue was collected beneath the rat skin. All collected tissues trimmed of connective tissues and fixed overnight in Zamboni's fixative (62). The tissue samples were later dehydrated in graded concentration of ethanol. The specimens were changed every 2 hours in 70% and 95% and 3 changes in absolute ethanol for 2 hours. After dehydration the specimens were cleared in xylene and subsequently embedded in paraffin wax at 55°C. Section of 6-7 μm thickness were cut on a microtome, and placed in water bath at 49°C. Thereafter, they were transferred onto microscopic slides, which were dried in an oven at 60°C for 30 min to enhance attachment of sections.

## II.2. Estimation of in vitro pancreatic insulin and glucagon release

In this experiment, 6 rats from untreated group were used. The pancreas was removed and placed in ice-cold PBS (phosphate buffered saline). The pancreas was trimmed free of adherent fat and connective tissue and cut into small fragments (0.1 g) and placed in 2 ml glass vials containing 1 ml of PBS and pre-incubated for 30 min in a water bath at 37°C in order to wash away any enzymes and hormones due to cutting of the tissues. After the pre-incubation period, the PBS solution was drained and the fragments were subsequently incubated for 1 h with different concentrations of visfatin and adiponectin ($10^{-12}$, $10^{-9}$ and $10^{-6}$ M). In order to examine the mechanism of action of adiponectin and visfatin pancreatic tissue fragments were also incubated with either atropine ($10^{-6}$ M); propranolol ($10^{-6}$ M); yohimbine ($10^{-6}$ M); or diltiazem ($10^{-6}$ M).

During the incubation period, each vial was gassed with 95% oxygen and 5% carbon dioxide every 10 min. At the end of experiment the tissues were removed, blotted, weighed and incubating solutions stored at -20°C for insulin and glucagon assay.

**II.2.a. Insulin assay**

Insulin was determined using DakoCytomation Insulin ELISA which was based on two monoclonal antibodies. Simultaneous incubation of sample (plasma or solution) and enzyme-labelled antibody in a microplate microwell coated with a specific anti-insulin antibody forms a complex. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by reaction with the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically. The inclusion of calibrators of known insulin concentration in the assay allows a calibration curve to be constructed from which the level of insulin in samples can be determined.

**II.2.b. Glucagon assay**

Glucagon was determined using Phoenix Enzyme immunoassay kit. The assay protocol started with adding of 50µL/well of standard sample positive control, 25µL primary antibody and 25µL biotinylated peptide to immunoplate and then incubation at room temperature for 2 hours. The immunoplate was washed 4 times with 350 µL/well of 1 x assay buffer followed by adding 100 µL/well of SA-HRP (streptavidin- horseradish peroxidase) solution then incubated at room temperature for 1 hour followed by washing the immunoplate 4 times with 350 µL/well of 1 x assay buffer. After that 100 µL/well of TMB (substrate solution) was added followed by incubation at room temperature for 1 hour using acetate plate sealer. Reaction terminated with 100 µL/well of 2N HCl added. The immunoplate was loaded to the reader and absorbance was read at 450 nm.

## II.3. Immunohistochemistry

Sections were de-paraffinized with xylene two times (5 min each) and then transferred into absolute ethanol two times (5 min each). After that the sections were incubated in 0.3% of hydrogen peroxide solution in methanol for 30 min to block the activity of endogenous peroxidase. The tissues were then hydrated in descending concentration of ethanol and washed 3 times in PBS (phosphate buffered saline) for 5 min each. After washing in PBS the tissue were marked around with a Dako pen to prevent solutions draining away from the tissue section.

The staining procedure was started by incubating the sections with blocking reagent for 30 min. After that the blocking reagent was drained off and appropriate dilution of the primary antibodies were applied and incubated at 4°C for 24 hr. On the following day the section were incubated at room temperature for 1 hr. The slides were then washed 3 times in PBS for (5 min each) and incubated with pre diluted biotinylated anti-guinea- pig or anti-mouse or anti-rabbit IgG (Sigma, St Louis, MO, USA) for 1 hour, then washed in PBS 3 'times (5 min each) and subsequently incubated in streptavidin peroxidase conjugate (Sigma) for 1 h. After a final wash in PBS 2 times (5 min each), the peroxidase activity was revealed by incubating the sections in 3,3diaminobenzidine tetrahydrochloride DAB (Sigma) in PBS for (5 min). The slides were then washed for 5 min under running tap water, and counterstained with haematoxylin for 15 seconds and washed briefly in tap water. Then they were differentiated in acid ethanol and washed for 5 min under running tap water, then dehydrated in ascending grades of ethanol, and subsequently cleared in xylene for longer time to dissolve the Dako pen mark. The tissues were subsequently mounted in DPX. Slides were examined under microscope and immunopositive areas of the tissue section were photographed .

## II.4. Immunofluorescenece

Isolated pancreatic tissues were retrieved, fixed and embedded in paraffin as described in section of tissue collection and tissue processing. Sections were de-paraffinized in xylene, hydrated in descending concentration of ethanol (3 min each) and washed 3 times in PBS for (5 min each). After washing in PBS, the tissue was marked around with a Dako pen to prevent solutions draining away from the tissue section. The staining procedure was started by incubating the sections with blocking reagent for 30 min. After that the blocking reagent was drained off and appropriate dilution of primary antibodies were applied and incubated at 4°C for 24 h. On the following day the sections were incubated at the room temperature for I hour. The slides were then washed 3 times in PBS (5 min each) and incubated with secondary antibodies conjugated to either FITC or TRITC for 1 hour and washed in PBS (3 times 5 min each). Sections were then mounted in using mounting media, and viewed and photographed under Nikkon Fluorescence microscope .

## II.5. Biochemical analysis

Using Beckman Coulter UniCel DxC 800 System(s) at Clinical Chemistry section at Al Ain Hospital the following plasma tests were performed (60,58):

1. Blood urea nitrogen (BUN) measured by means of an enzymatic conductivity rate method.
2. Creatinine (CRE) measured by means of the Jaffe rate method.
3. Alkaline phosphatase (ALP) measured by a kinetic rate method using a 2-amino-2-methyl-1-propanol (AMP) buffer.
4. Alanine aminotransferase (ALT) plasma, aspartate aminotransferase (AST), Amylase (AMY), lactic dehydrogenase (LDH) and Gamma¬glutamyl transferase (GGT) measured by an enzymatic rate method.
5. Cholesterol (CHOL), HDL cholesterol, Triglycerides (TG) and phosphorus (PHOS) by a timed-endpoint method.
6. Sodium by indirect potentiometry utilizing two glass sodium electrodes (one acts as the reference electrode).
7. Albumin (ALB) by means of a bichromatic digital endpoint methodology using bromcresol purple (BCP) reagent.
8. Total protein (TP) measured by means of a rate biuret method.
9. Chloride measured by indirect potentiometry methode utilizing a solid state chloride electrode in conjunction with a glass sodium reference electrode (Cl).

## II.6. Electron microscopy studies

Pancreas fragments were trimmed free of adherent connective tissue and cut into small pieces of 2 mm and fixed overnight in freshly prepared Karnovsky fixative (Karnovsky, 1965). They were later washed 3 times in 0.1 M Phosphate buffer and postfixed in 1% osmium tetroxide for 1 hour. After post-fixation, they were washed 5 times in buffer and dehydrated. Dehydration of tissue was done in ethanol series, with a single change of 15 min in 30%, 50%, 70%, 95% and 4 changes in absolute ethanol for 15 min each. After the dehydration step, the samples were cleared in propylene oxide (2 changes of 15 min each) and transferred to 1: 1 mixture of propylene oxide and resin for 1 hour and later transferred to 1: 2 mixtures of propylene oxide and resin. One hour after the 1:2 mixture, the tissues were infiltrated with resin overnight. The samples were later embedded in resin using moulds; polymerisation of the resin block was completed in 24 hours at 55°C. The blocks were trimmed and 1μm semi-thin sections were cut with glass knives on an ultra microtome (Reichert Ultracut S, USA) and sections were transferred onto drops of water on a microscope slide using watchmaker's forceps. The slides were stained with toludine blue and observed under electron microscope to locate the islets of Langerhans .

## II.7. Immunoelectron microscopy

The pancreas was trimmed free of adherent fat and connective tissue and cut into small pieces of 2 $mm^3$ and fixed overnight in freshly prepared mix of (4% paraformaledhyde and 0.05 % glutaraldehyde for at 4°C Thereafter, they were washed 3 times in 0.1 M phosphate buffer and dehydrated in concentrated ethanol series with a single change of 20 min in 70%, 95%, 95%. After the dehydration step, the tissue samples were transferred to a 1: 1 mixture of 95% ethanol and resin (LR White) for 1 hour for infiltration and later transferred into LR White resin over night at 4°C. In the next day, the tissues were transferred from resin to gelatin capsules under UV lamp for 24 hours to allow for polymerization. The tissue blocks were further trimmed for ultra-thin sectioning. Ultra-thin sections were cut at a thickness of 80 nm by a diamond knife. The sections were transferred onto 300 mesh copper grid by a wire loop. The grids were dried on a

filter paper and stained with uranyl acetate for 30 min and washed 5 times in filtered distilled water. To further enhance the contrast, the sections were stained in lead citrate for 3 min and washed 3 times in filtered distilled water. The dried grids were observed on transmission electron microscope (Philips CM 10, Eindhoven, Holland) and photographed.

## II.8. Quantification of gene expression in pancreatic tissues

The relative quantification of genes was performed using real-time PCR. Total RNA was extracted from rat pancreatic tissues using RiboPure (Applied Biosystems, Forster City, CA, USA) according to the manufacturer's recommendations. The concentration and the purity of the RNA sample was determined by measuring absorbance at 260 nm and 280 nm using the NanoDrop 1000A Spectrophotometer. cDNA synthesized using High Capacity cDNA Reverse Transcription Kit with RNase.

Gene expression assays using TaqMan labeled primers and probes for the requested target genes (insulin receptor gene, pancreatic and duodenal homeobox 1, GLP-1R gene, heme oxygenase Homx-1, glutathione peroxidase 2, HSP70) was done. The endogenous control was beta-actin. By using an endogenous control as an active reference, normalizing quantification of a messenger RNA target for differences in the amount of total RNA added to each reaction. Relative quantification was performed using real-time PCR. In real-time PCR assays, the progress of the PCR as it occurs was monitored. Data were collected throughout the PCR process rather than at the end of the PCR process. Data was analyzed by creating an RQ Study document using SDS software for 7500 ABI Prism Sequence Detection system.

## II.9. Statistical analyses

All data were expressed as mean ± SD. Student's T-test was used to analyze the significance of differences between mean values of different groups. A p value of less than 0.05 was considered to be statistically significant .

# RESULTS

# I. Effect of adiponectin and visfatin on metabolic parameters in STZ-induced and GK rats

## I.1. Body weight

Figure (1) shows the pattern of weight gain or loss in different animal models of diabetes during 14 week treatment with 10 ng/kg body weight of adiponectin and visfatin. A continual trend of weight gain was observed in normal Wistar rats treated with either adiponectin or visfatin (1.A). However, diabetic Wistar rats lost weight continuously up to the 6th week of treatment before slightly gaining weight till the end of the experiment at week 8 (1.B).GK rats gained weight continuously throughout the course of the experiment irrespective of whether they treated with either adipopnectin or visfatin (1.C).

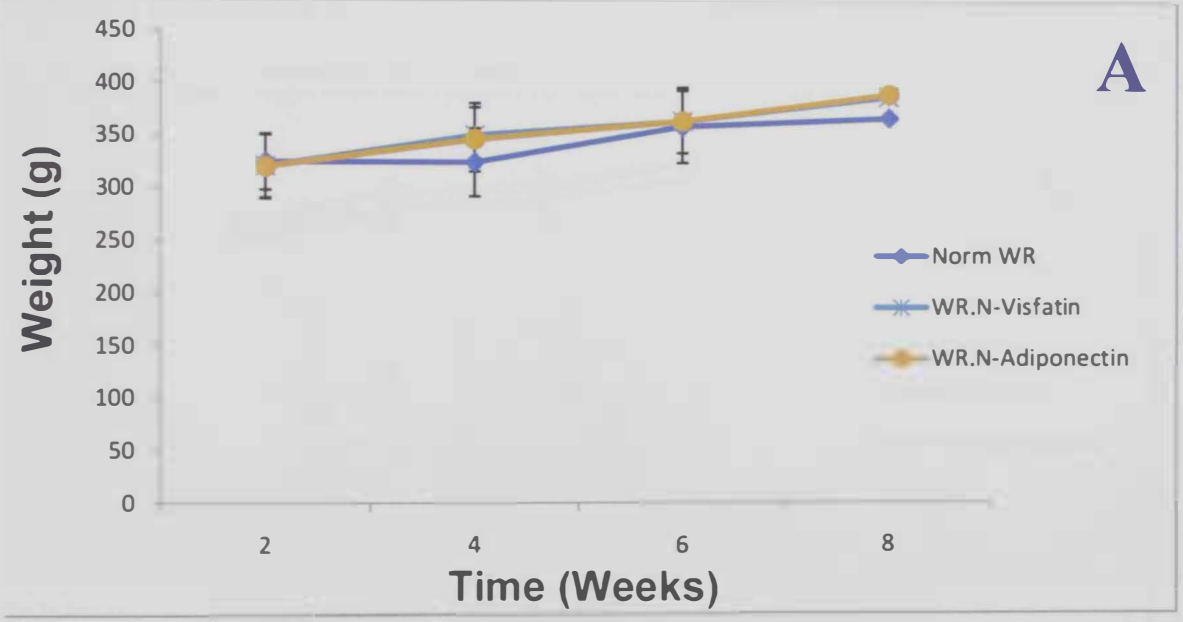



Figure (1.A) shows the effect of visfatin and adiponectin on body weight gained or lost in normal WR. No significant difference in body weight was observed in treated and control rats (Data are mean ±SD, n=5).

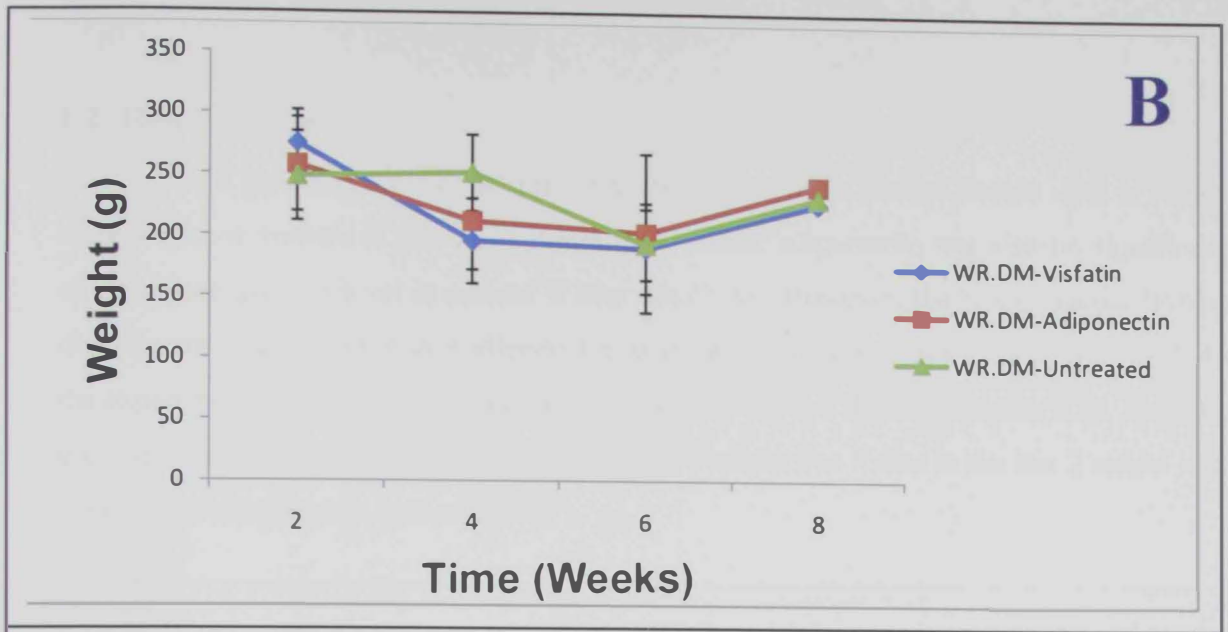



Figure (1.B) shows the effect of visfatin and adiponectin on body weight gained or lost in diabetic WR. No significant difference in body weight was observed in treated and control rats (Data are mean ±SD, n=5).

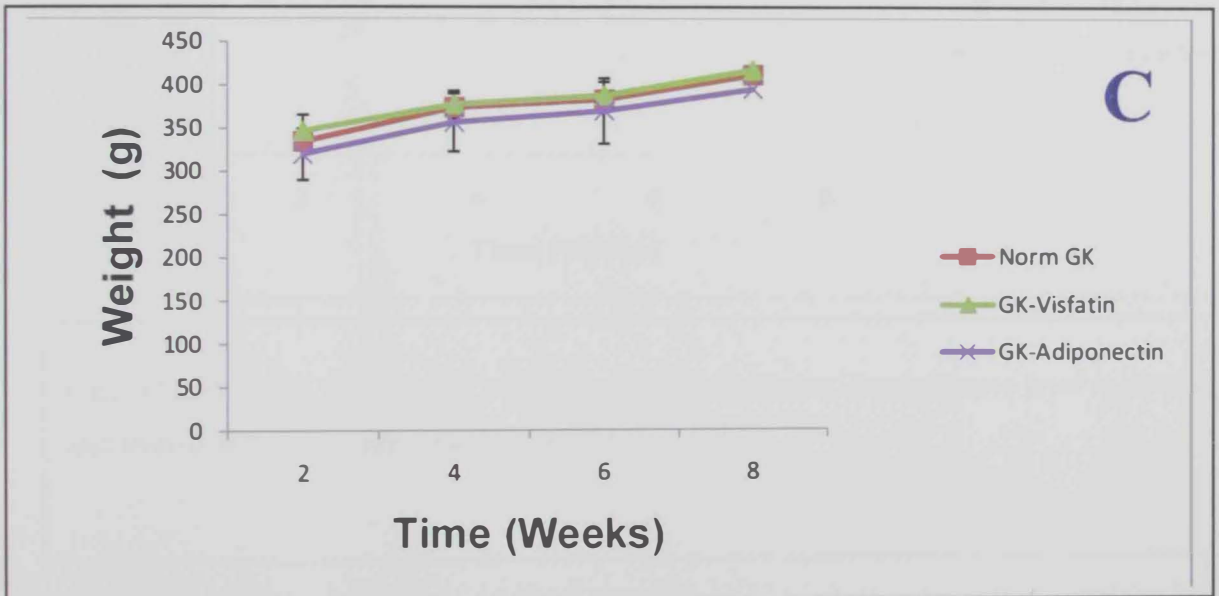



Figure (1.C) shows the effect of visfatin and adiponectin on body weight gained or lost in GK rats. No significant difference in body weight was observed in treated and control rats (Data are mean ±SD, n=5)

## I.2. Blood glucose

Blood glucose levels of normal, diabetic Wistar and GK rats treated with or without adiponectin or visfatin is shown in figure (2). Neither adiponectin nor visfatin significantly altered blood glucose level in normal Wistar rats (2.A). However, the blood glucose level of diabetic rats treated with either adiponectin or visfatin was significantly reduced in week 4 of the experimental period before increasing towards the end of the experiment (2.B). The blood glucose level of GK rats treated adiponectin and visfatin was lower in the last 2 weeks of the experiment compared to control (2.C).

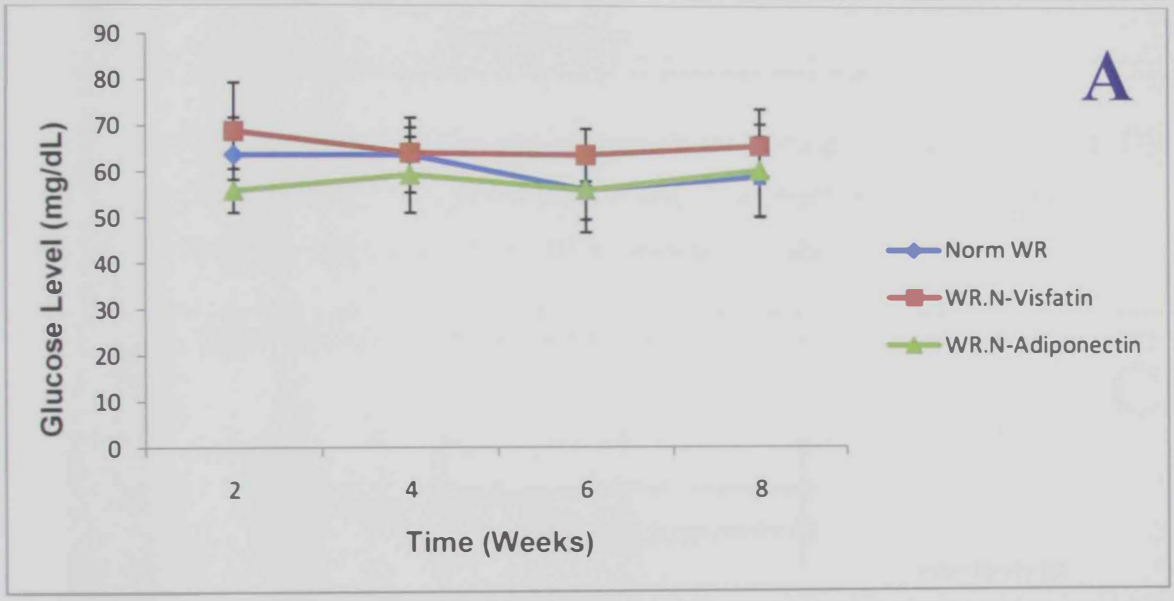



Figure (2.A) shows the effect of visfatin and adiponectin on blood glucose level in normal and treated WR. (Data are mean ± SD, n=5)

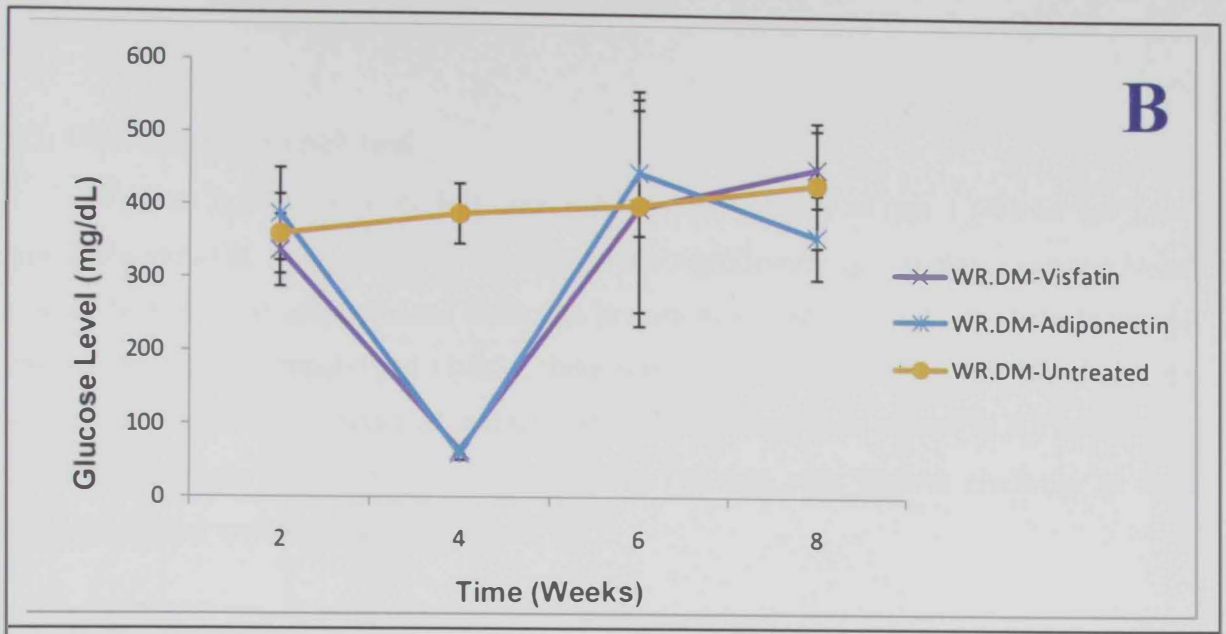



Figure (2.B) shows effect of visfatin and adiponectin on blood glucose level of treated DM-WR. Glucose level decreased sharply in week 4 after treatement with visfatin and adiponectin. (Data are mean ± SD, n=5). DM: Diabetes mellitus

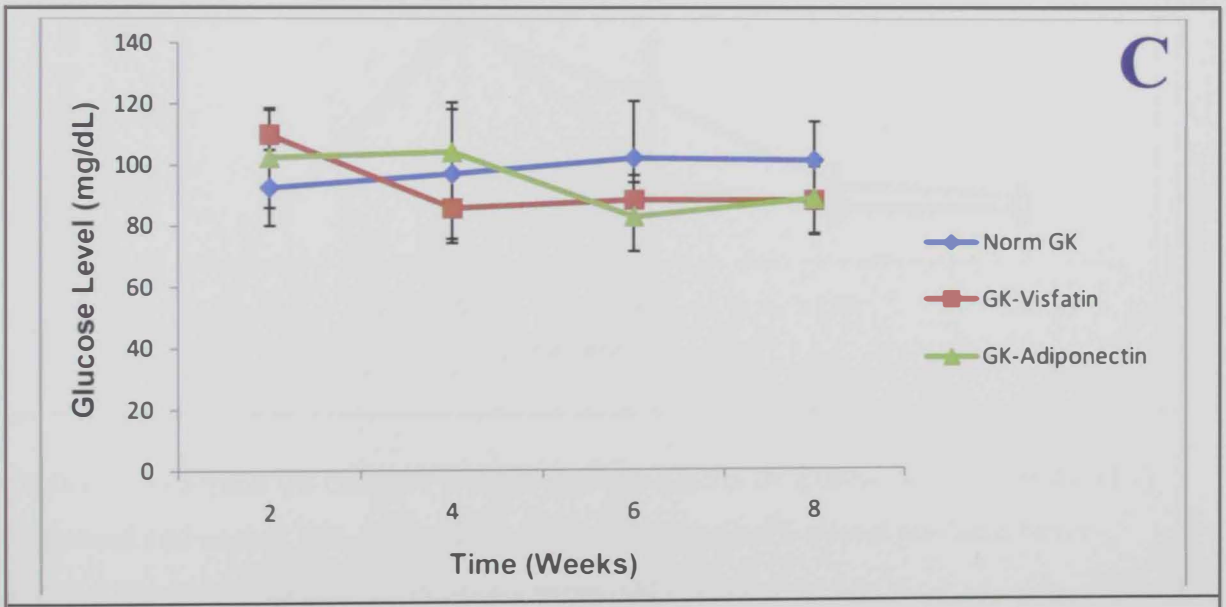



Figure (2.C) representative the effect of visfatin and adiponectin on blood glucose level of GK treated rats. Glucose level in treated rats appear lower compared to control. (Data are mean ±SD, n=5).

## I.3. Glucose tolerance test

Figure (3) shows glucose tolerance test in animal models of type 1 (Wistar rats ) and type 2 diabetes(GK rats). Adiponectin and visfatin significantly ($p < 0.0003$) reduced blood glucose level 60 min after glucose challenge in normal Wistar rats (3.A). In diabetic Wistar rats treated with adiponectin and visfatin, there was no significant difference in blood glucose level after treatment with either adiponectin or visfatin compared to control (3.B). In GK rats, blood glucose was significantly (p 0.005) lower 120 min after glucose challenge in both adiponectin and visfatin treated rats (3.C).

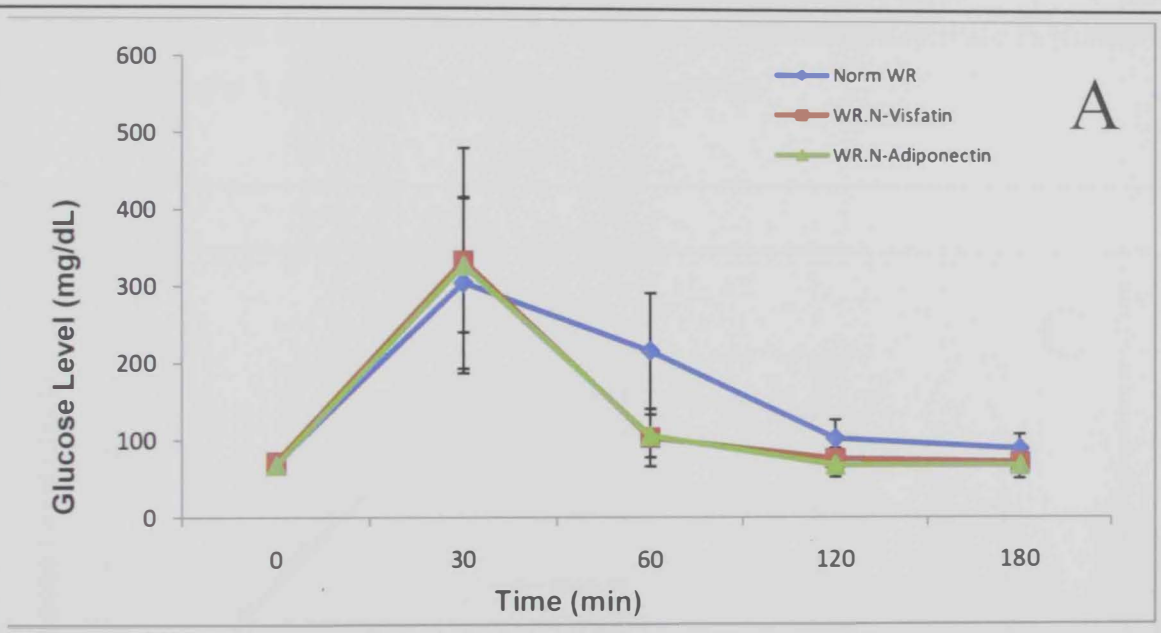



Figure (3.A) depicts the effect of visfatin and adiponectin on glucose tolerance test (GTT) of normal and treated WR. Note that visfatin and adiponectin-treated rats have better glucose handling after 60 min. (Data are mean ±SD, n=5)

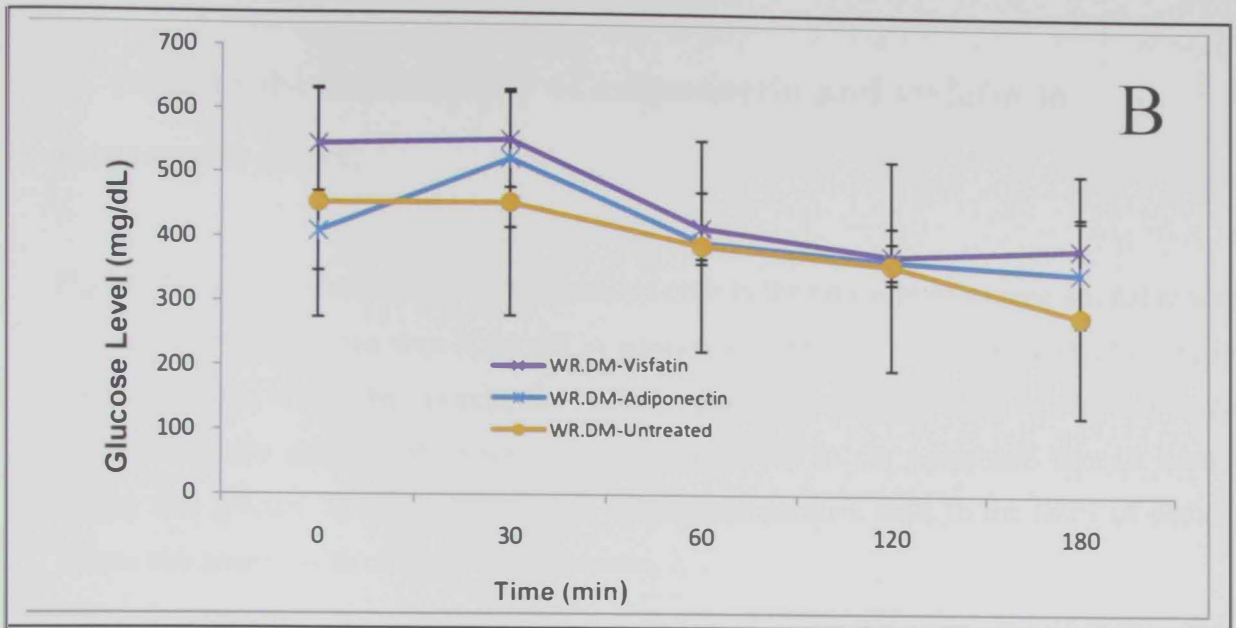



Figure (3.B) shows the effect of visfatin and adiponectin on glucose tolerance test (GTT) of treated DM- WR (Data are mean ± SD, n=5). There was no significant difference in glucose handling between the 3 groups of rats. DM: Diabetes mellitus

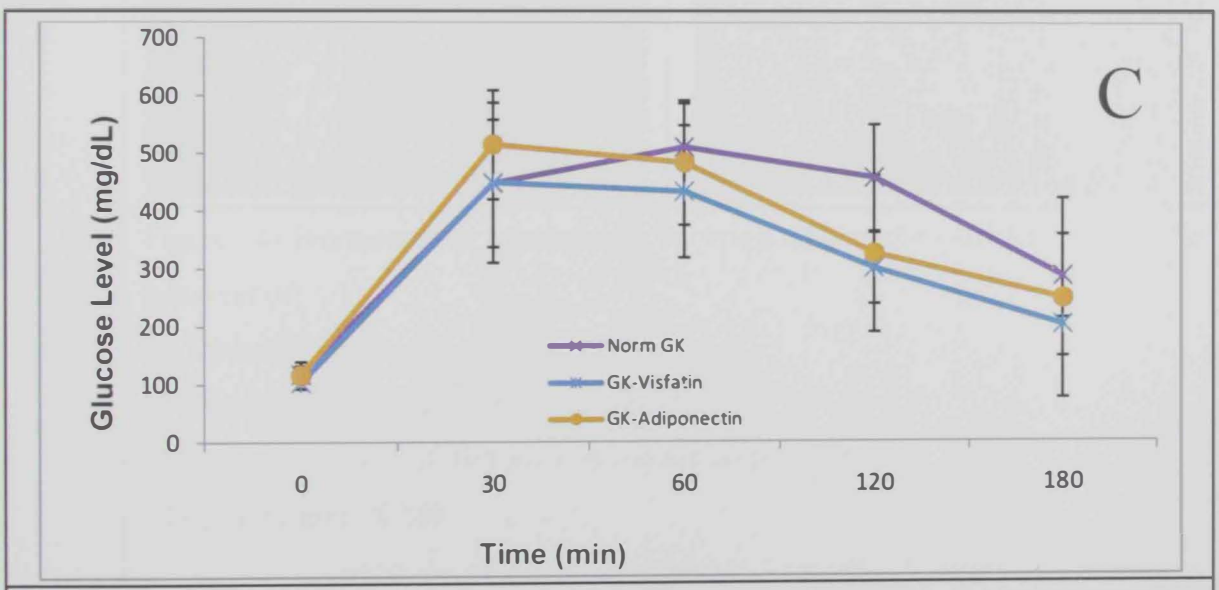



Figure (3.C) depicts the effect of visfatin and adiponectin glucose tolerance test (GTT) of normal and GK treated rats. Note that visfatin and adiponectin-treated rats have better glucose handling after 120 min. (Data are mean ±SD, n=5)

## II. Immunohistochemistry of adiponectin and visfatin in pancreatic islets.

Figures (4) and (5) shows adiponectin-positive cells in the pancreas of various animal models of diabetes. Adiponectin was observed in pancreatic islet cells of normal and adiponectin-treated GK rats, where they occupy the central region. The administration of adiponectin did not significantly enhance the expression of adiponectin in the pancreatic islet of normal Wistar rats (Figure 6). Figure (4) shows adiponectin-positive cells in the islets of diabetic Wistar rats treated with or without adiponectin.

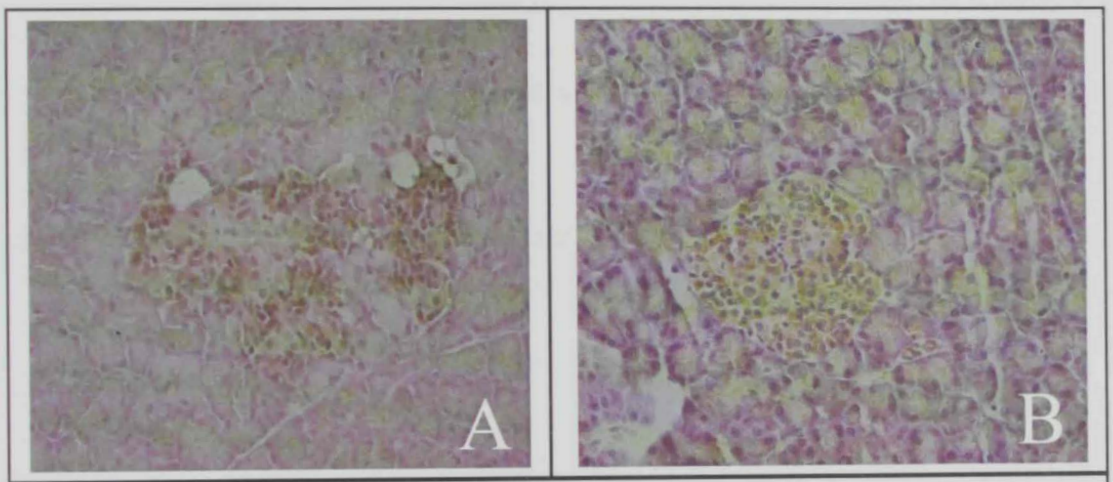



Figure (4) representative micrographs showing adiponectin-positive cells in the pancreas of:

A- Diabetic Wistar rats

B- Diabetic Wistar treated with adiponectin

*Note that the islets of diabetic rats contain adiponectin.

**Magnification: X 200**

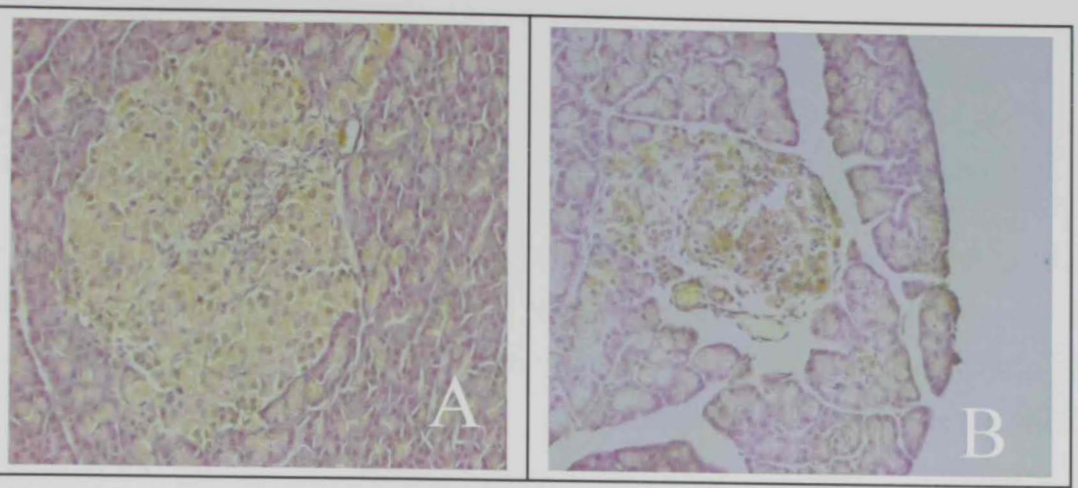



Figure (5) representative micrographs showing adiponectin-positive cells in the pancreas of:

A- Normal GK rats

B- GK treated with adiponectin

*Note that the expression of adiponectin is stronger in treated rats compared to control.

**Magnification: X 200**

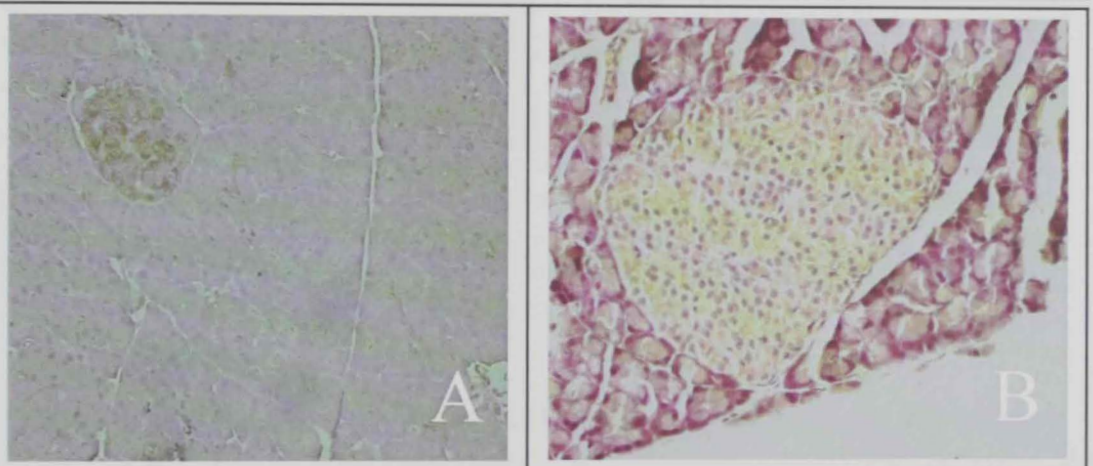



Figure (6) representative micrographs showing adiponectin-positive cells in the pancreas of:

A- Normal Wistar rats

B- Normal Wistar rats treated with adiponectin.

*Note that the expression of adiponectin is stronger in treated normal Wistar rats compared to control.

**Magnification: X 200**

Figures (7) and (9) shows visfatin-positive cells in the pancreas of various animal models of diabetes. Visfatin was observed in pancreatic islet cells of normal and visfatin-treated GK rats, where they occupy the central region. The administration of visfatin did not significantly enhance the expression of visfatin in the pancreatic islet of normal Wistar rats (Figure8). Figure (9) shows visfatin-positive cells in the islets of diabetic Wistar rats treated with or without visfatin

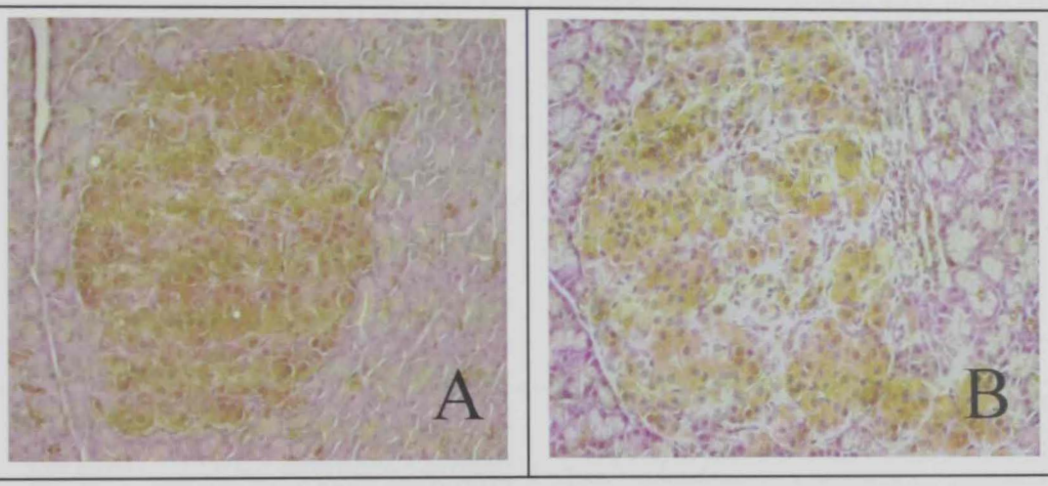



Figure (7) representative micrographs showing visfatin-positive cells in the pancreas of:

A- Normal GK rats

B- GK treated with visfatin

*Note that visfatin is present in the islets of GK rats and increased tremendously after treatment.

**Magnification: X 200**

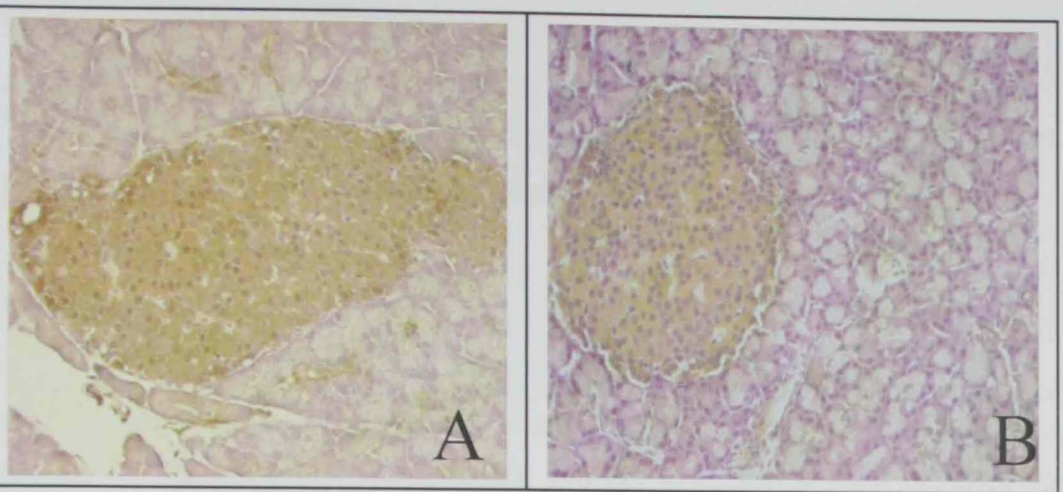



Figure (8) representative micrographs showing visfatin-positive cells in the pancreas of:

A- Normal Wistar rats

B- Normal Wistar rats treated with visfatin

Note that visfatin is present in the pancreas of Wistar rats. Treatment did not significantly increase islet expression of visfatin.

**Magnification: X 200**

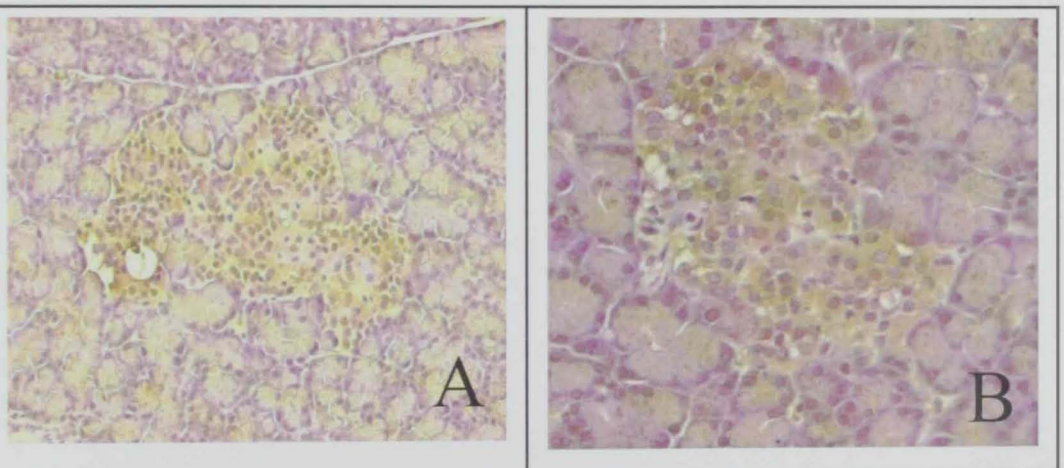



Figure (9) representative micrographs showing visfatin-positive cells in the islet of:

A- Diabetic Wistar rats

B- Diabetic Wistar treated with visfatin

*Not that a reduction in the degree of expression of visfatin is discernible in the islet. **Magnification: X 200**

# III. Immunofluorescence studies

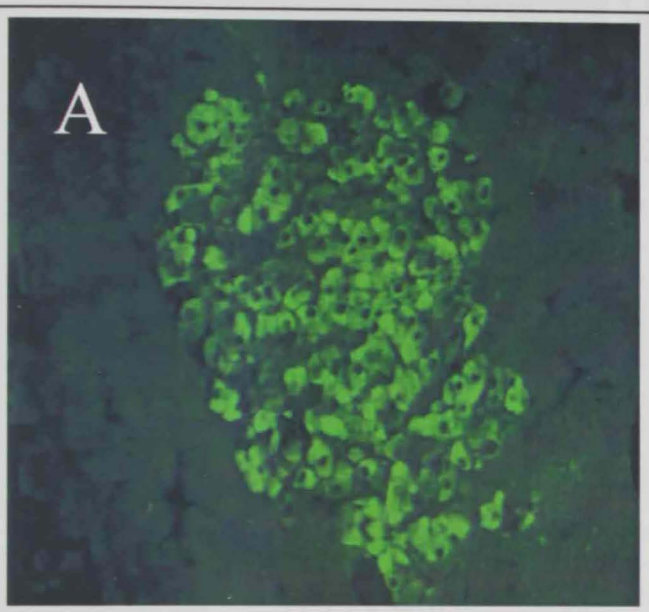
## III.1. Immunofluorescence studies of adiponectin in pancreatic islets of various animal model of diabetes.

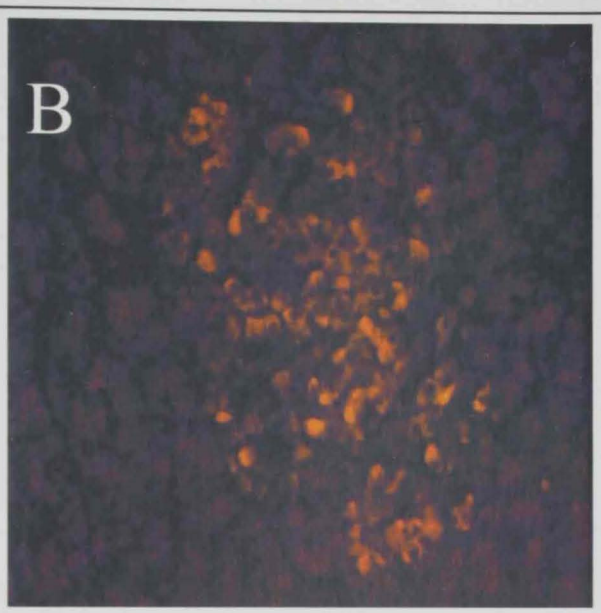
### III.1.a. Adiponectin and insulin

Immunofluorescence study shows that many islet cells in the pancreas of GK rats contain adiponectin. Many islet cells contain both adiponectin as well as insulin (Figure 10). Treatment of GK rats did not significantly increase the number of adiponectin containing cells in pancreatic islets (Figure 11).

In a similar way, normal and adiponectin-treated Wistar rats contain large numbers of adiponectin-positive cells (Figure 12). Treatment of normal Wistar rats with adiponectin increases the degree of co-localization between insulin and adiponectin (Figure 13).

The number of adiponectin-immunoreactive cells decreased significantly after the onset of diabetes in Wistar rats (Figure 14). However, treatment of diabetic Wistar with adiponectin significantly increased the number of insulin positive cells in the pancreatic islets of diabetic rats (Figure 15).



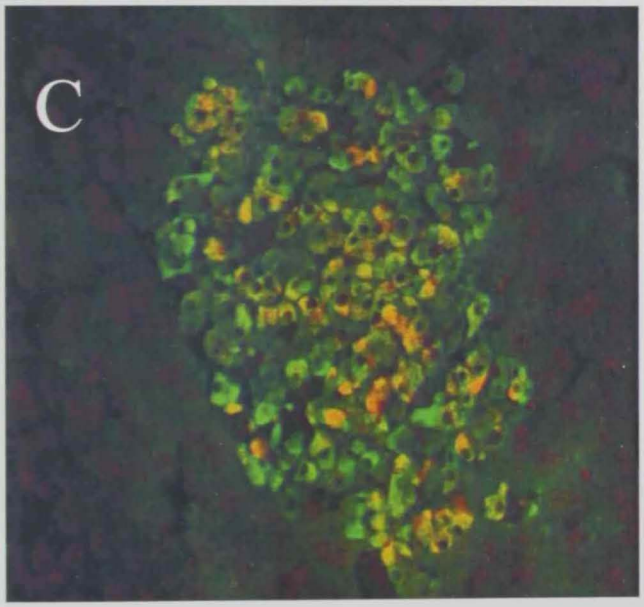




Figure (10) representative micrographs showing insulin positive- (Green) and adiponectin positive- (Red) cell in pancreatic islets of control GK rats.

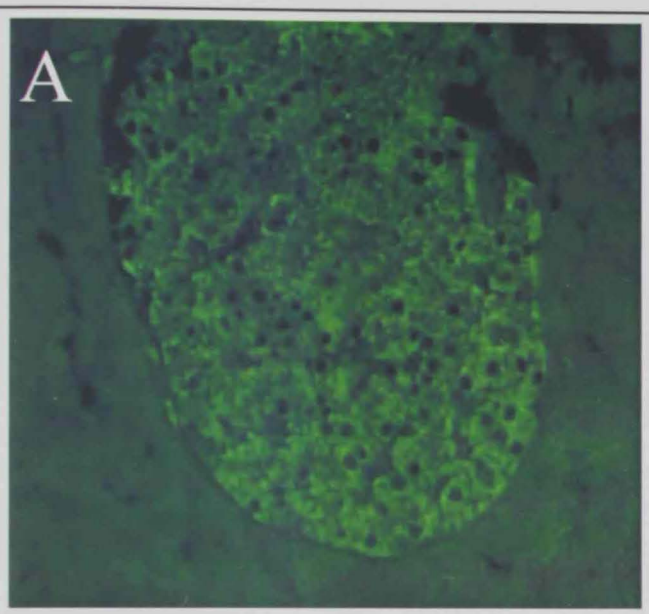
A- Insulin (Alone)

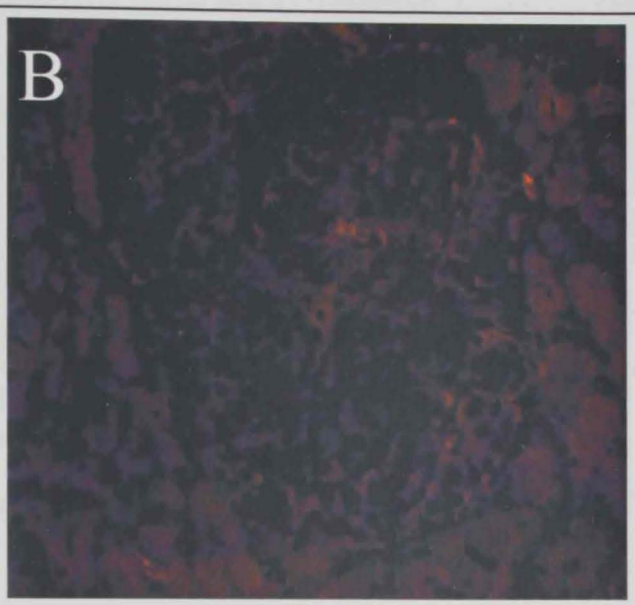
B- Adiponectin (Alone)

C- Merged

Not that many islet cells contain both adiponectin and insulin.

**Magnification: X 200**



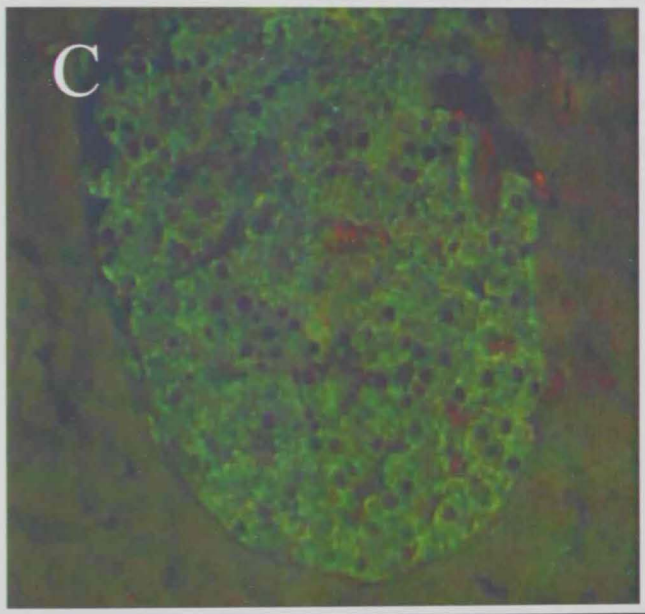



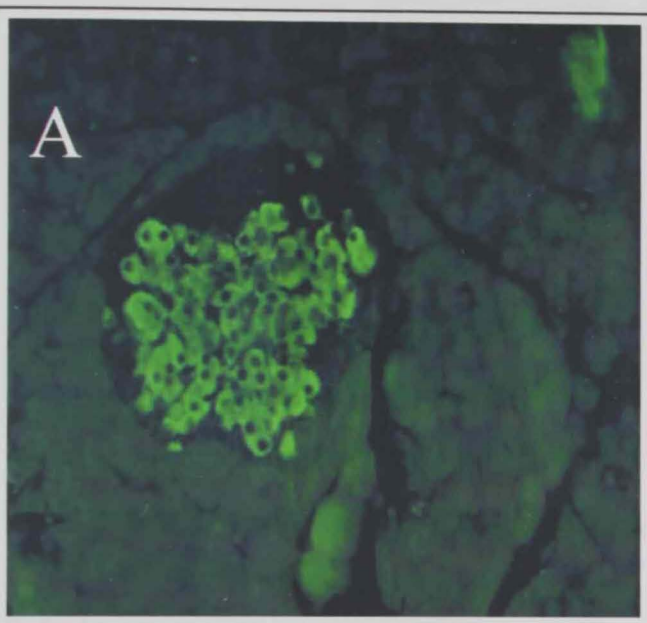

Figure (11) representative micrographs showing insulin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of GK rats treated with adiponectin.

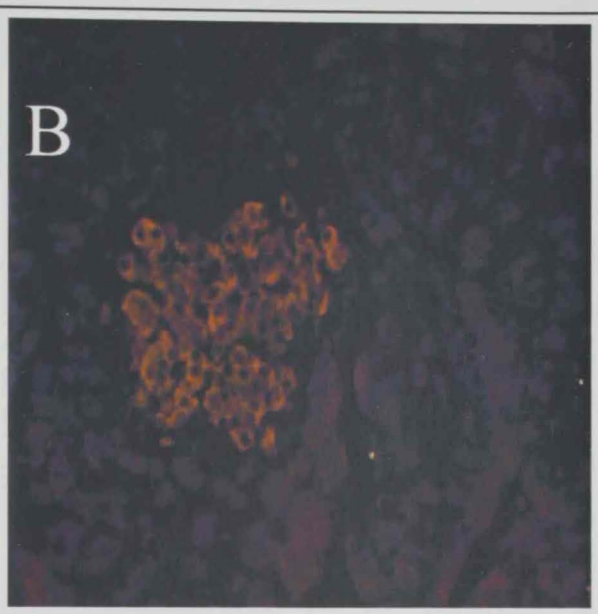
A- Insulin (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**



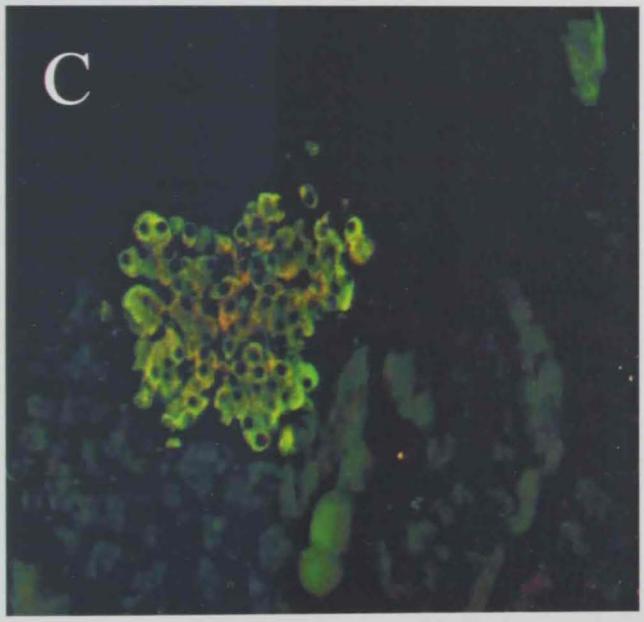




Figure (12) representative micrographs showing insulin (Green) and adiponectin-positive cell (Red) in pancreas islets of normal Wistar rats.

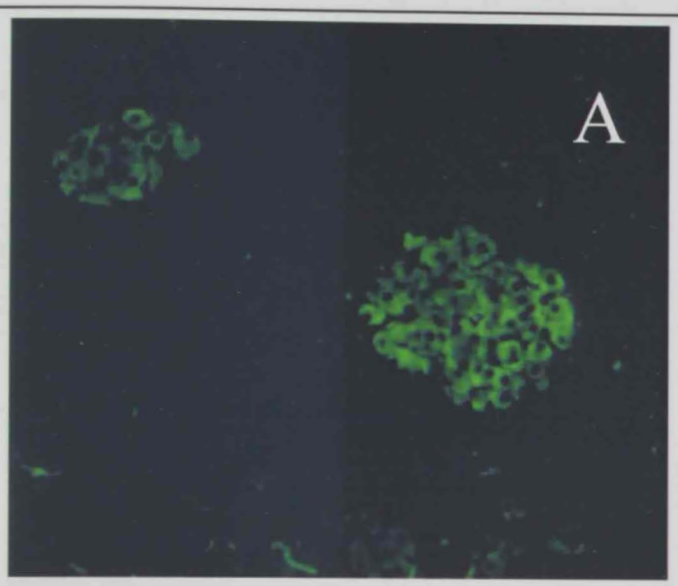
A- Insulin (Alone)

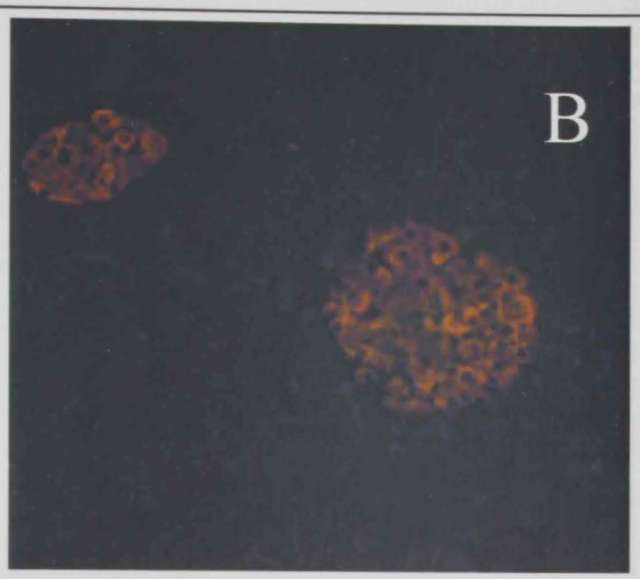
B- Adiponectin (Alone)

C- Merged

*Note that Insulin colocalizes with adiponectin in islet cells.

**Magnification: X 200**



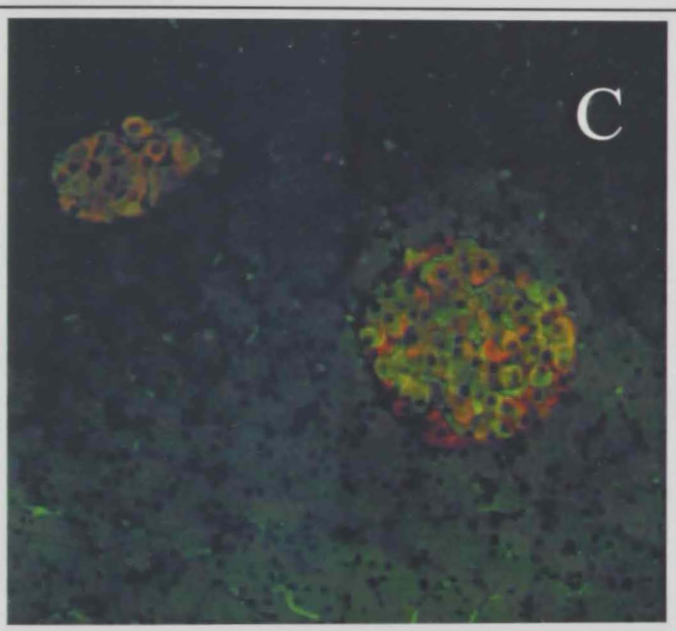




Figure (13) representative micrographs showing insulin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal Wistar rats treated with adiponectin.

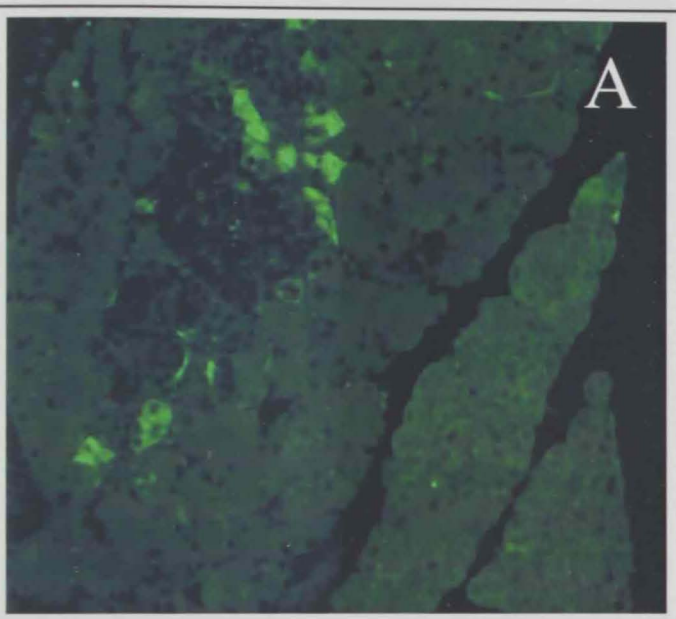
A- Insulin (Alone)

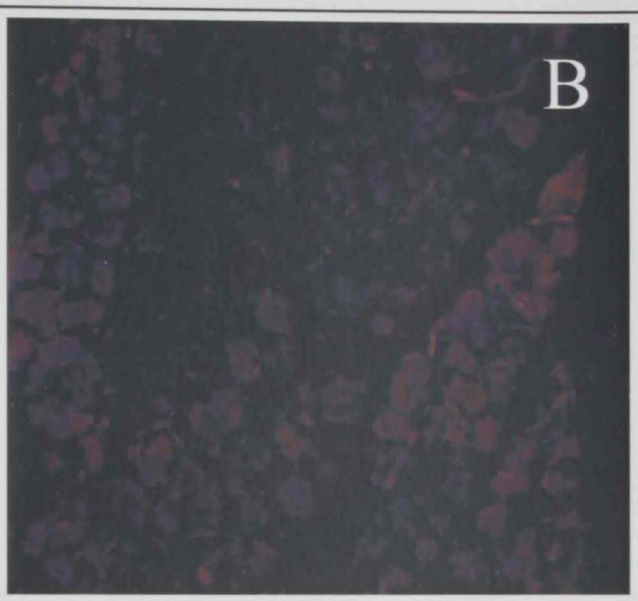
B- Adiponectin (Alone)

C- Merged

*Note that the number of adiponectin-positive cells increased after treatment. The degree of co-localization of adiponectin and insulin is enhanced.

**Magnification: X 200**



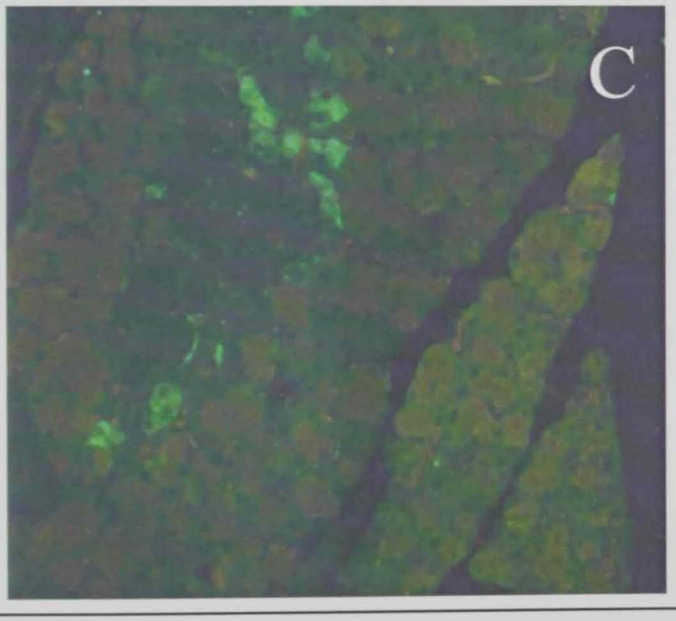



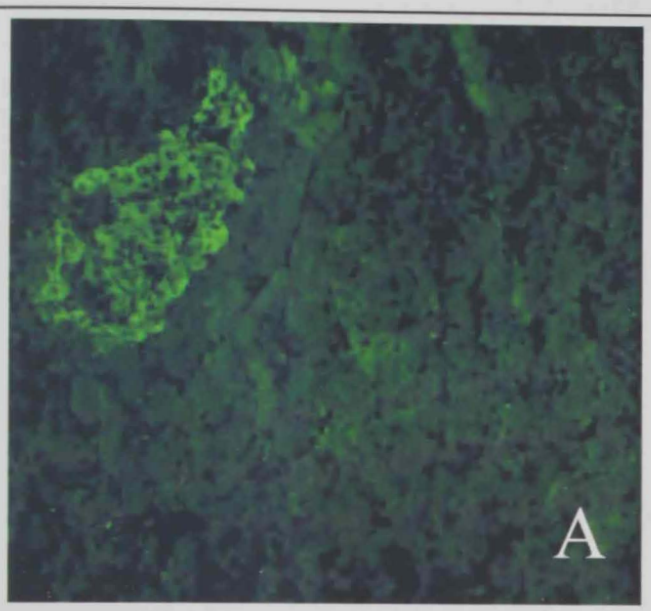

Figure (14) representative micrographs showing insulin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic untreated Wister rats.

A- Insulin (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**





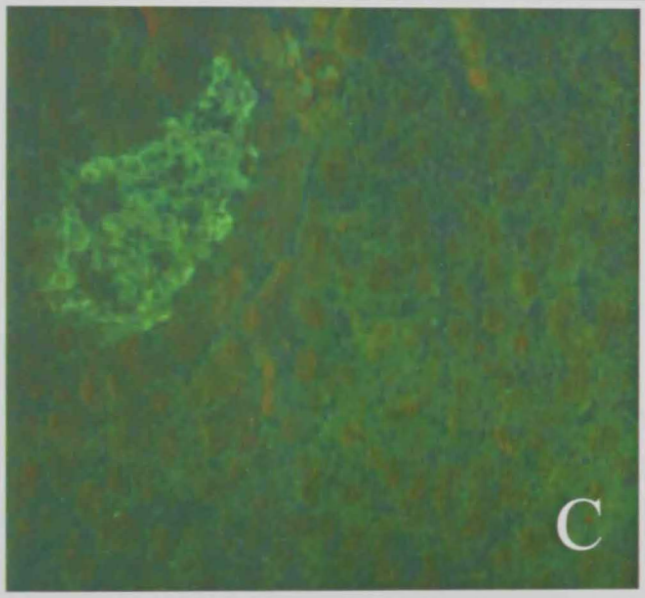



Figure (15) representative micrographs showing insulin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of treated diabetic Wistar rats.

A- Insulin (Alone)

B- Adiponectin (Alone)

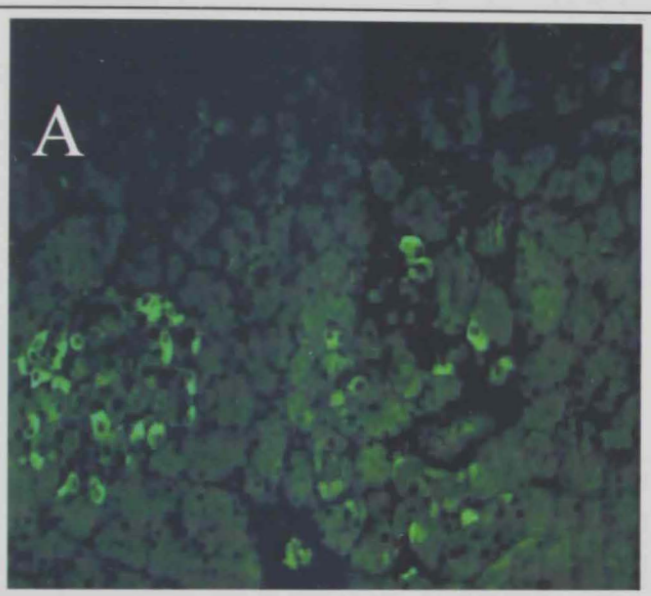
C- Merged

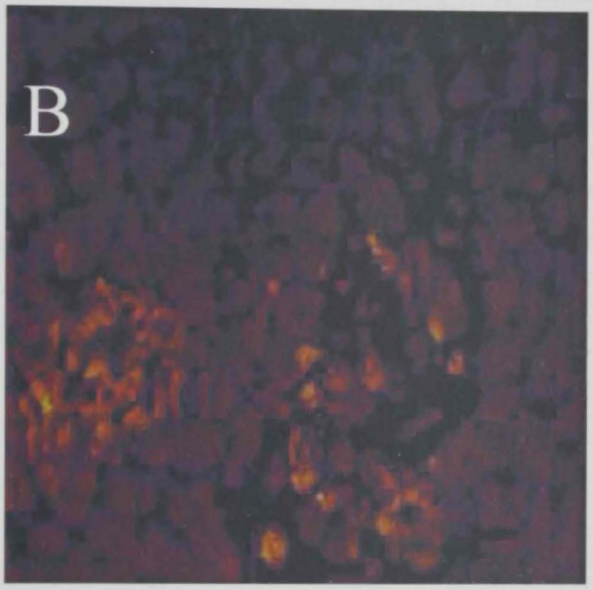
*Note that treatment with adiponectin increased the number of insulin positive cells.

**Magnification: X 200**

### III.1.b. Adiponectin and glucagon

The pancreatic islets of GK rats contain glucagon, which appear to be more in number compared to Wistar rats (Figure 16). Adiponectin also co-localizes with glucagon in pancreatic islets of GK rats treated with adiponectin (Figure 17). Adiponectin is present in pancreatic islets of normal Wistar rats (Figure18,21) but decreased significantly after the onset of diabetes (Figure 19). Treatment of diabetic Wistar rats with adiponectin enhanced cellular co-localization between adiponectin and glucagon (Figure 20).



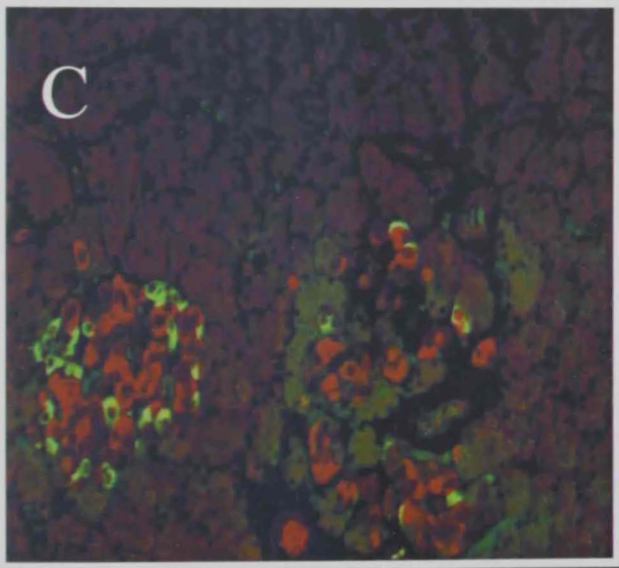




Figure (16) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal GK rats.

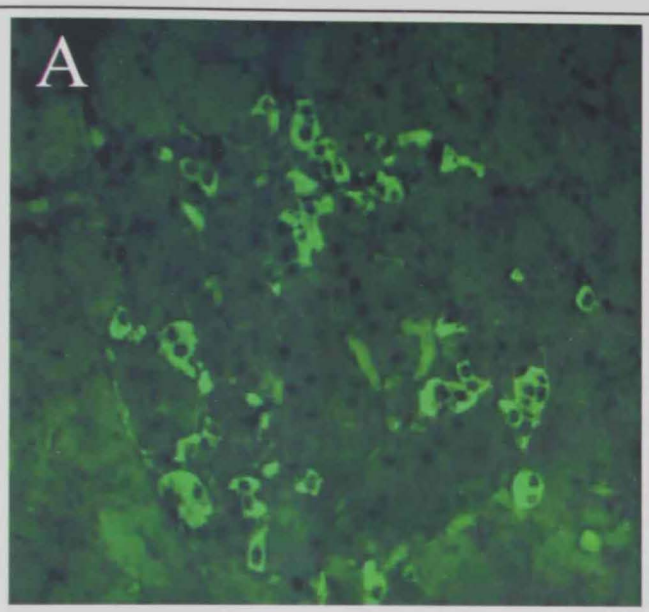
A- Glucagon (Alone)

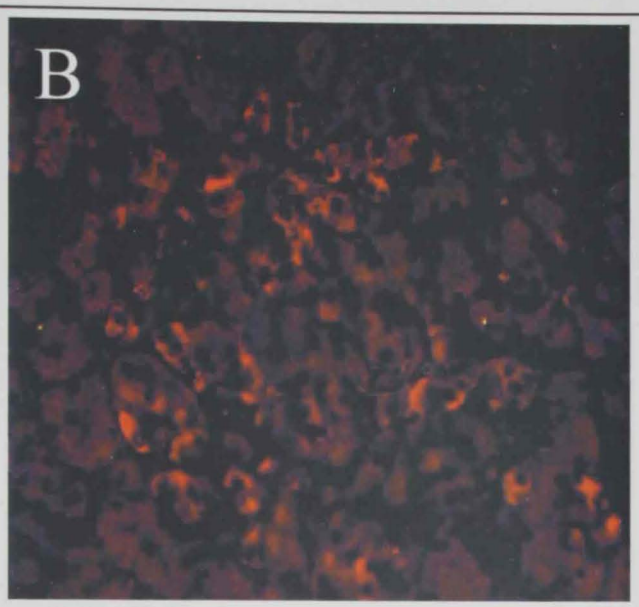
B- Adiponectin (Alone)

C- Merged.

*Note that Glucagon and adiponectin-positive cells are present in the islets of GK rats

**Magnification: X 200**



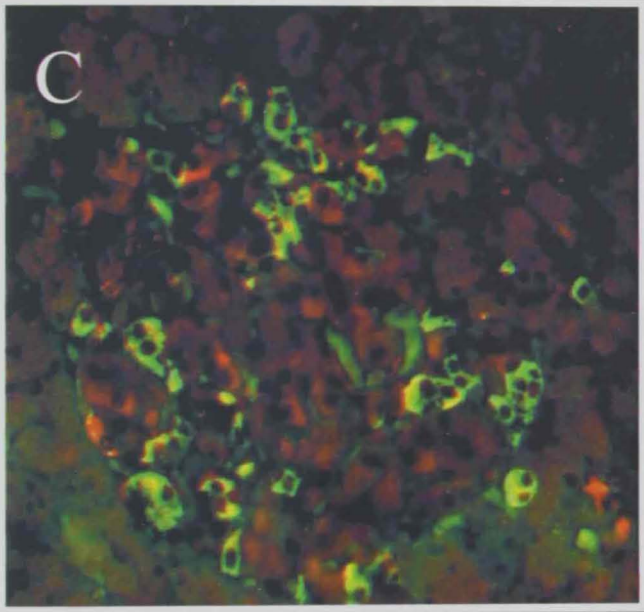




Figure (17) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of GK rats treated with adiponectin.

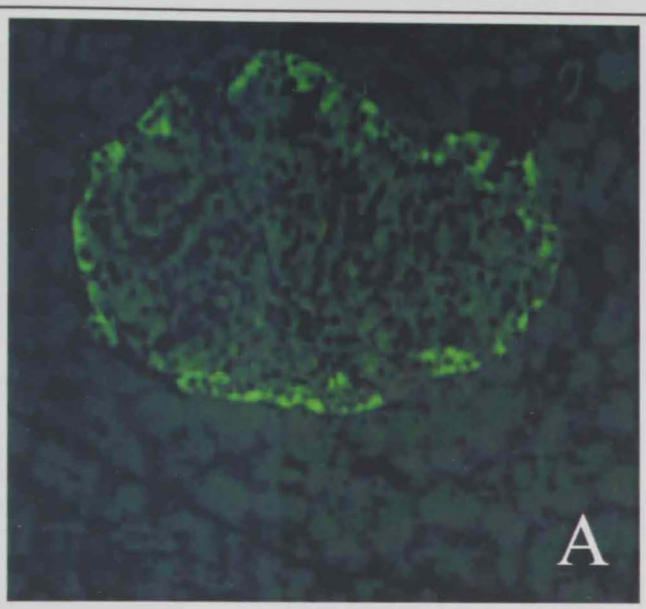
A- Glucagon (Alone)

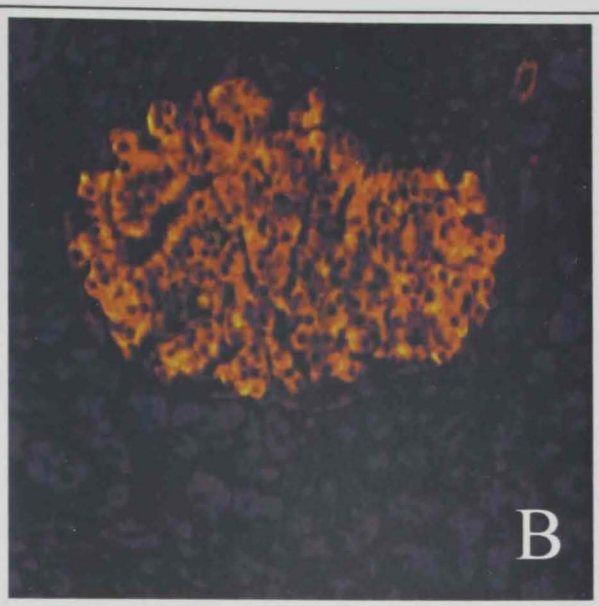
B- Adiponectin (Alone)

C- Merged.

*Note that some islet cells contain both adiponectin and glucagon

**Magnification: X 200**



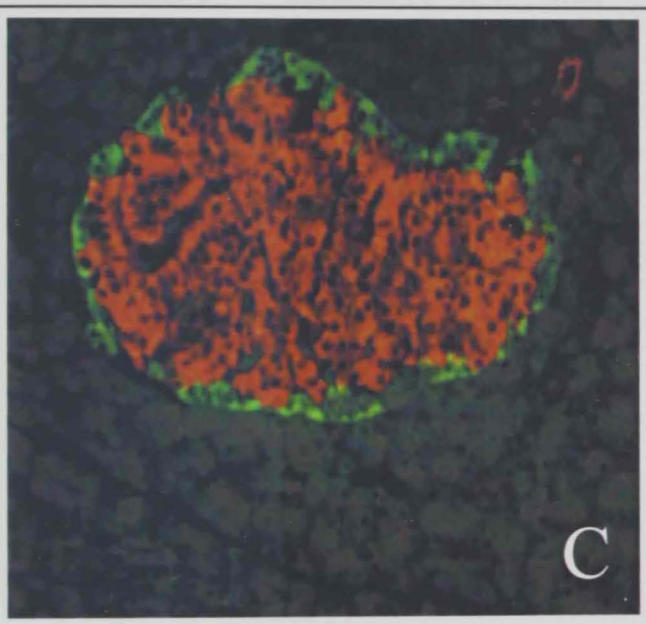




Figure (18) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of Normal Wister rats.

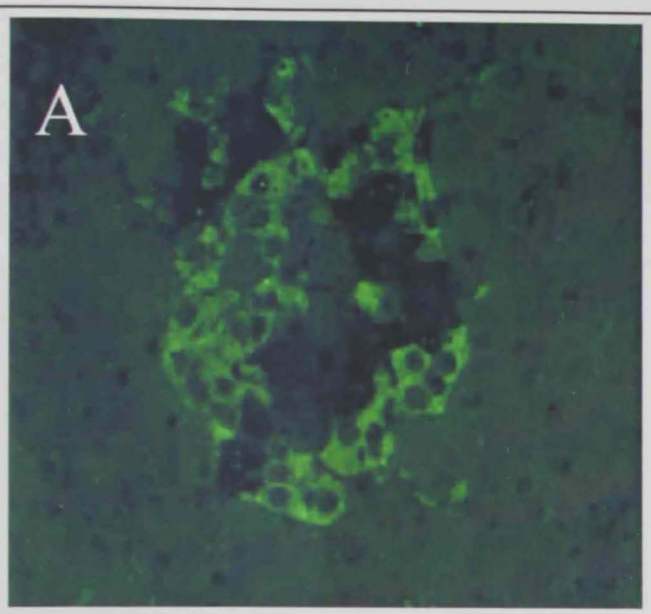
A- Glucagon (Alone)

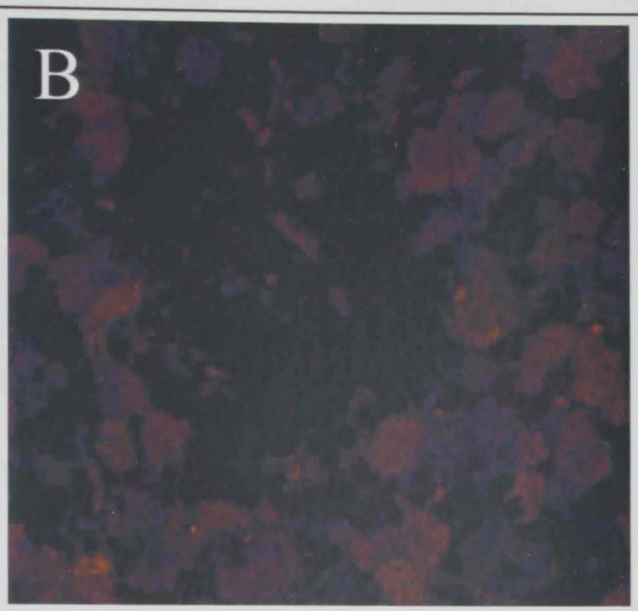
B- Adiponectin (Alone)

C- Merged

*Note that Islet cells contain large number of adiponectin.

**Magnification: X 200**



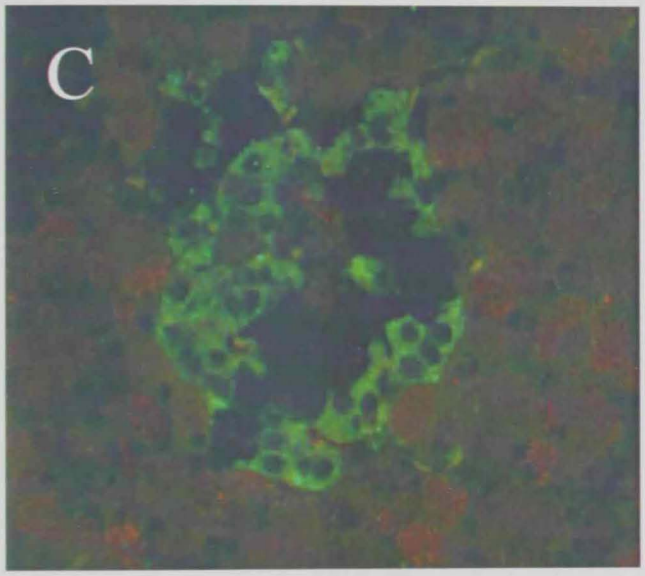




Figure (19) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic untreated Wistar rats.

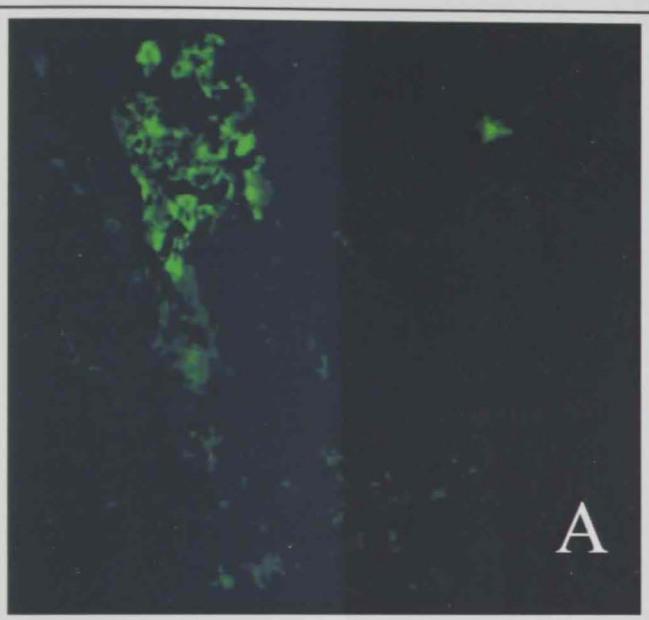
A- Glucagon (Alone)

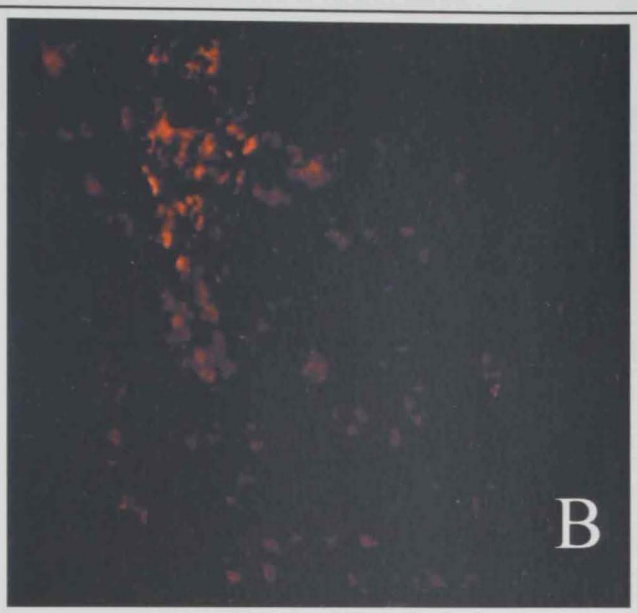
B- Adiponectin (Alone)

C- Merged

*Note that the number of adiponectin-positive cells is reduced in diabetes

**Magnification: X 200**



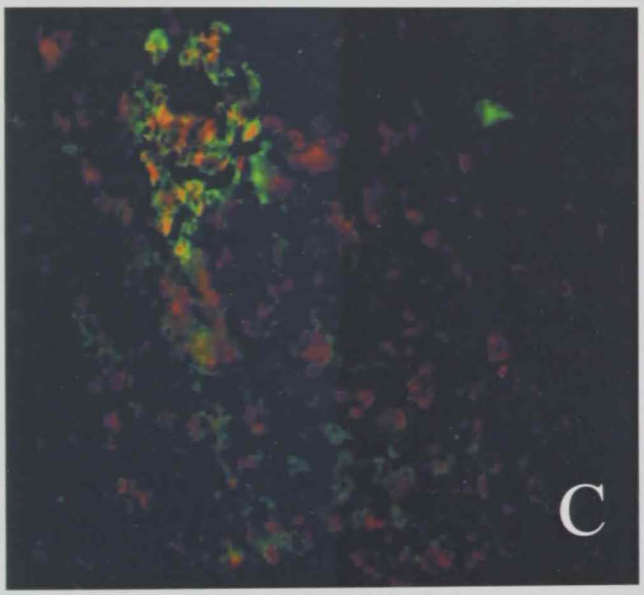




Figure (20) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic treated Wistar rats.

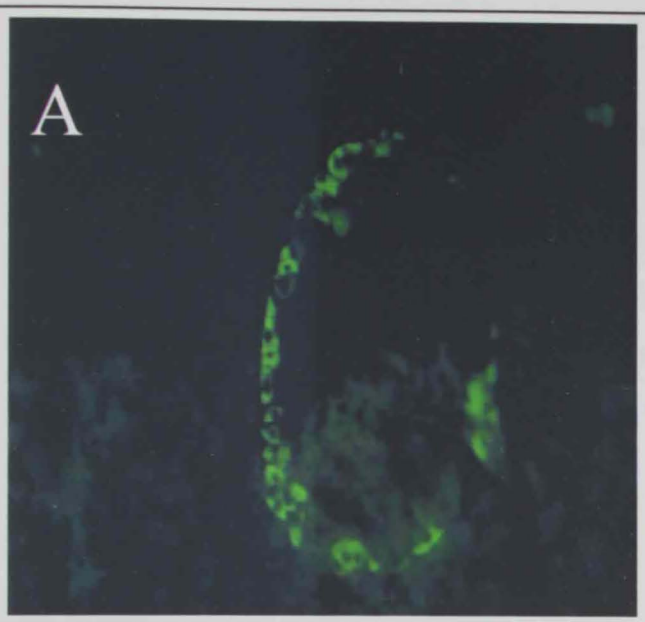
A- Glucagon (Alone)

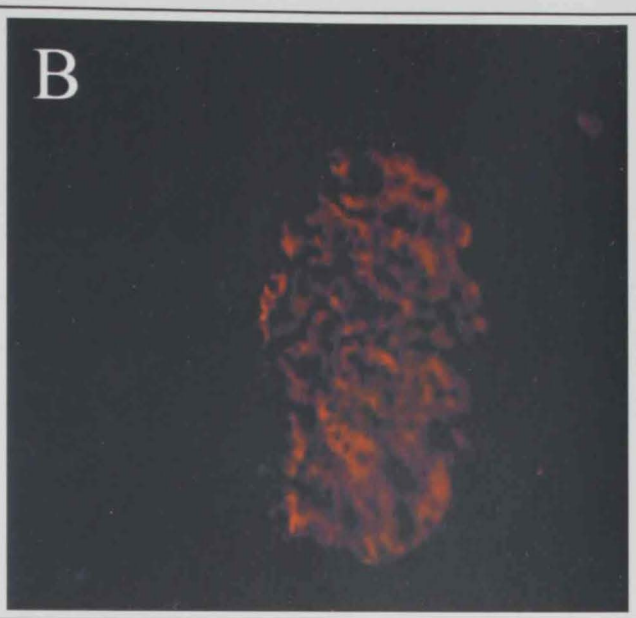
B- Adiponectin (Alone)

C- Merged

Note that there is co-localization of adiponectin with glucagon in islets of treated rats

**Magnification: X 200**



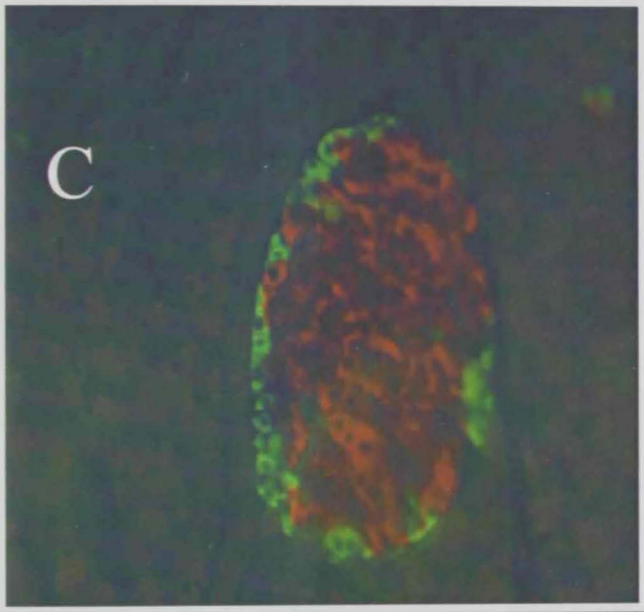




Figure (21) representative micrographs showing glucagon positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal Wistar rats treated with adiponectin.

A- Glucagon (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**

### III.1.c. Adiponectin and somatostatin

Adiponectin does not co-localize with somatostatin in pancreatic islet cell of GK rats (Figure 22). Treatment of GK rats with adiponectin reduced the number of somatostatin positive cells in the islet of Langerhans (Figure 23). In normal Wistar rats, administration of adiponectin increased the expression of adiponectin and reduced the tissue expression of somatostatin (Figure 24, 25). Treatment of diabetic Wistar rats restored the expression of adiponectin (Figure 26). Many cells in the islets of diabetic Wistar rats contain both adiponectin and somatostatin (Figure 27).

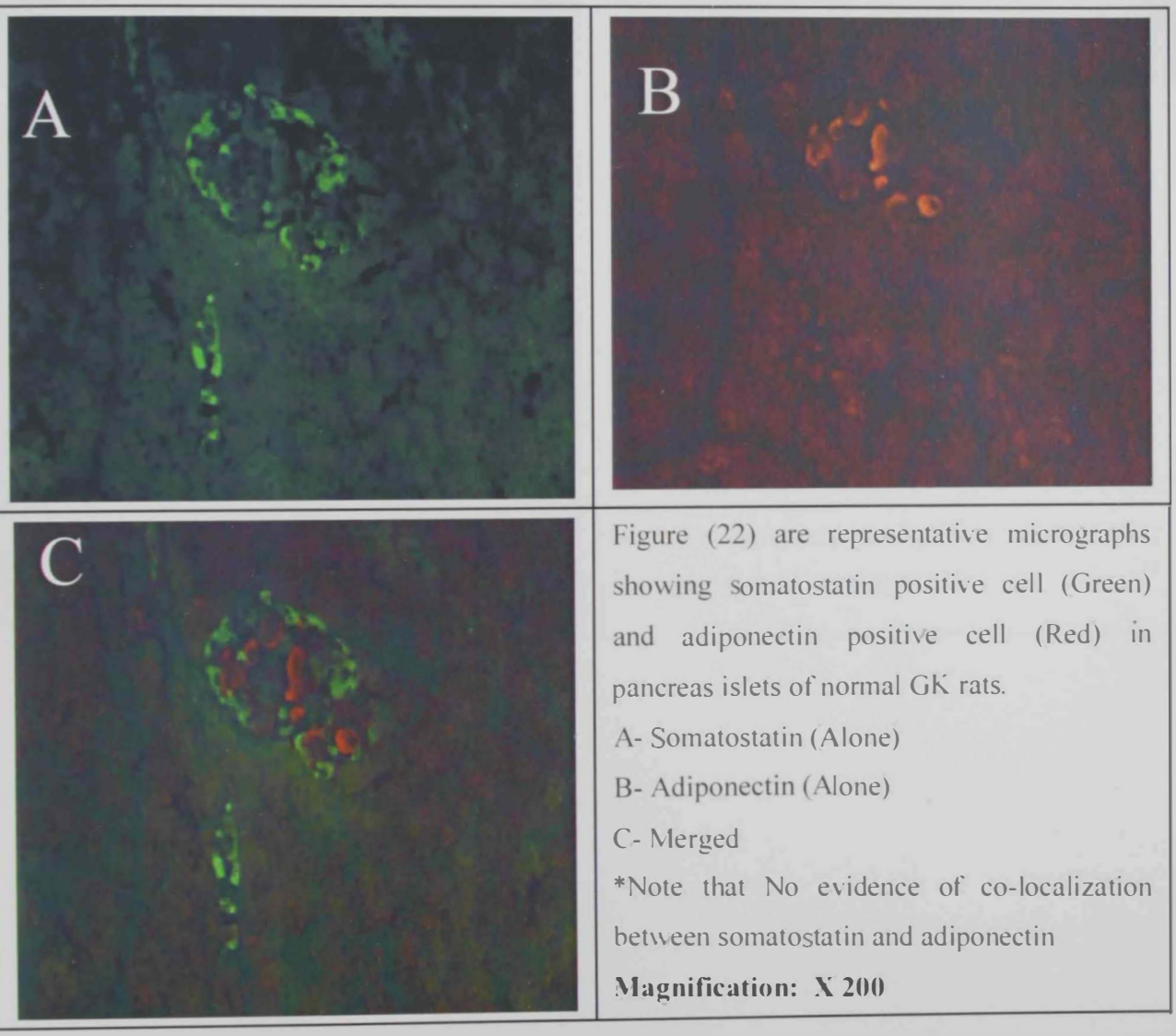



Figure (22) are representative micrographs showing somatostatin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal GK rats.

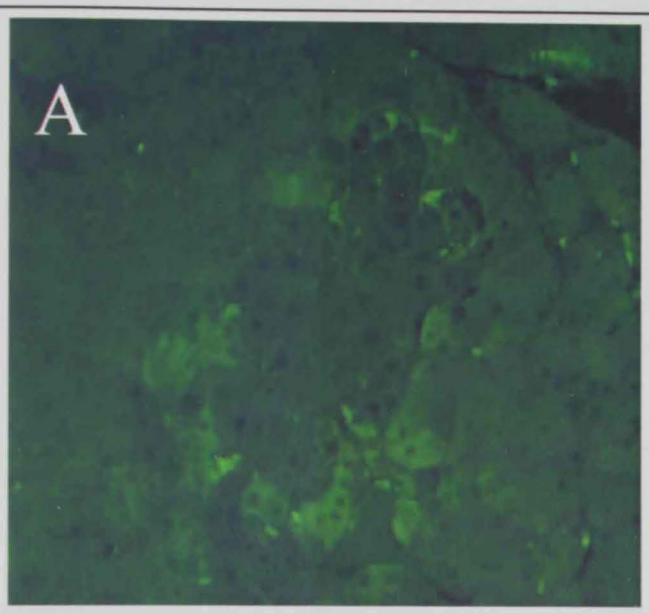
A- Somatostatin (Alone)

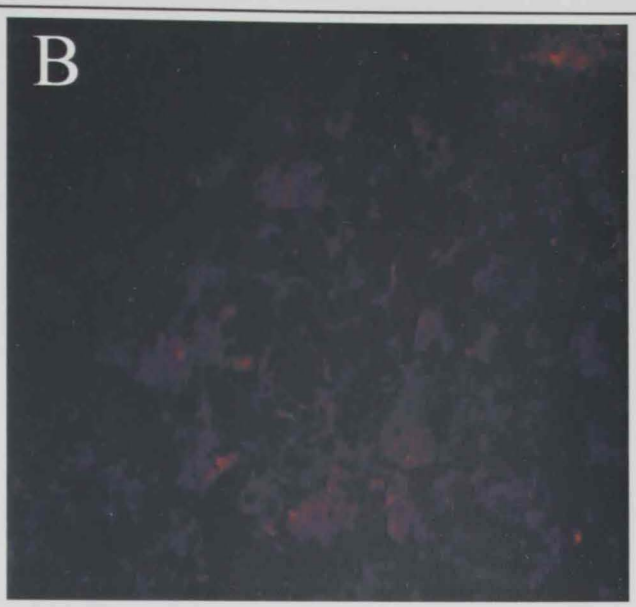
B- Adiponectin (Alone)

C- Merged

*Note that No evidence of co-localization between somatostatin and adiponectin

**Magnification: X 200**



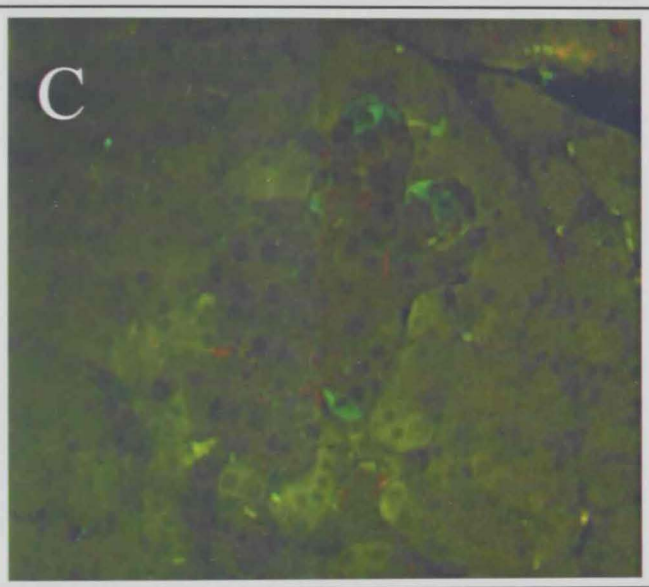




Figure (23) representative micrographs showing somatostatin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of GK-treated rats.

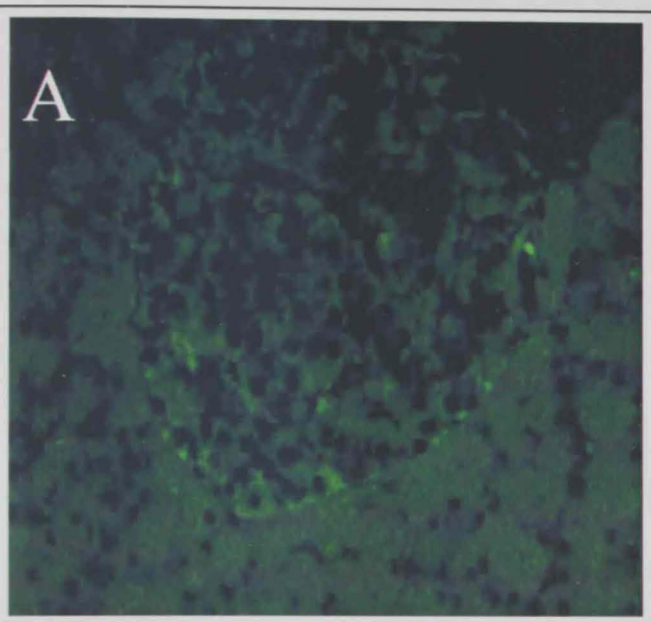
A- Somatostatin (Alone)

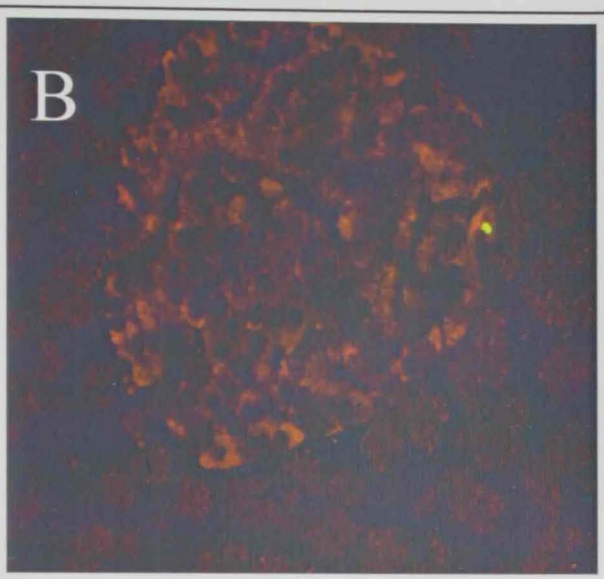
B- Adiponectin (Alone)

C- Merged

*Note that the number of somatostatin-positive cells has decreased in adiponectin-treated rats.

**Magnification: X 200**



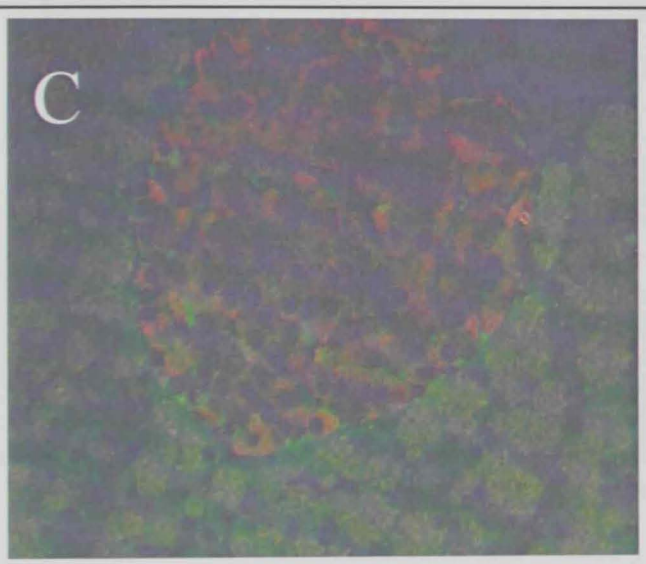




Figure (24) representative micrographs showing somatostatin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal-treated Wistar rats.

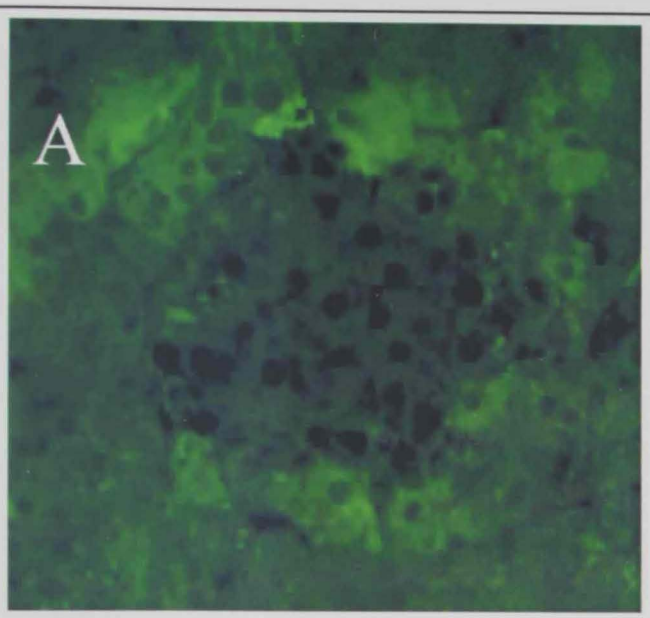
A- Somatostatin (Alone)

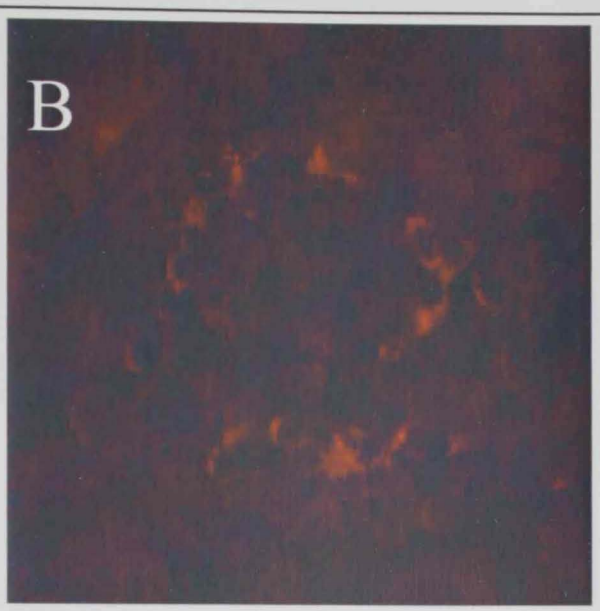
B- Adiponectin (Alone)

C- Merged

*Note that no co-localization between adiponectin and somatostatin.

**Magnification: X 200**



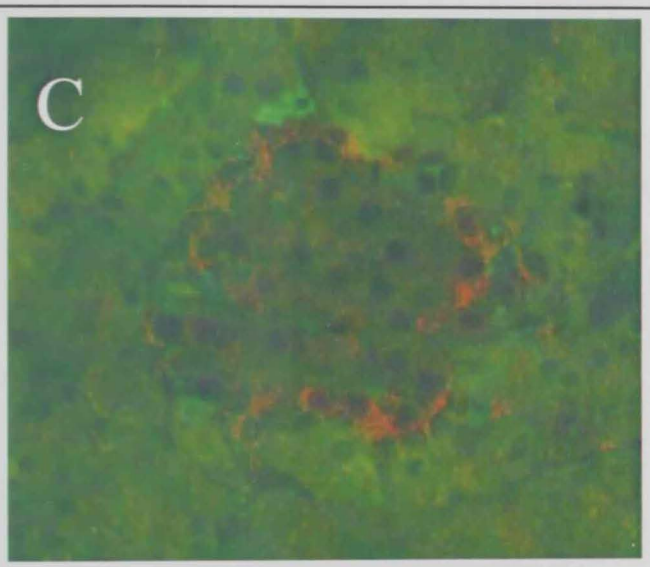




Figure (25) representative micrographs showing somatostatin (Green) and adiponectin-positive cell (Red) in pancreas islets of normal untreated Wistar rats.

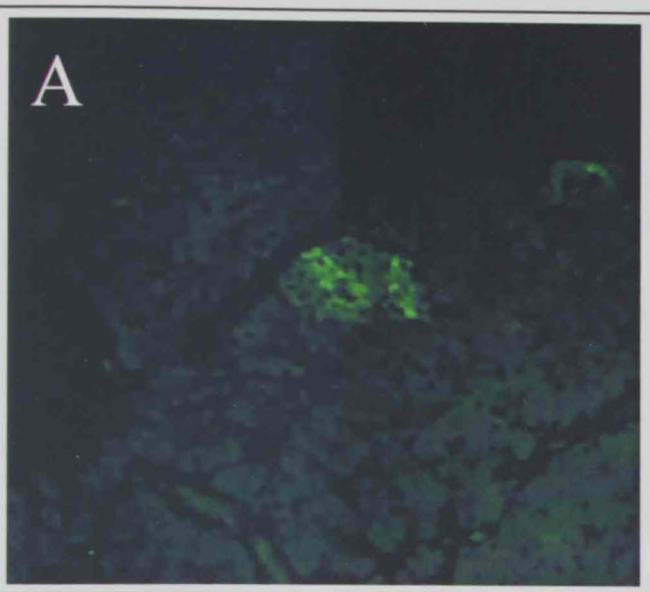
A- Somatostatin (Alone)

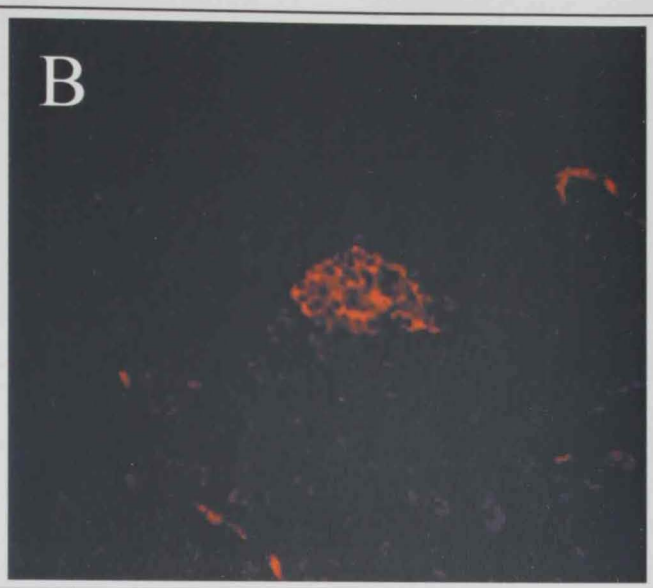
B- Adiponectin (Alone)

C- Merged

*Note that no co-localization between adiponectin and somatostatin.

**Magnification: X 200**



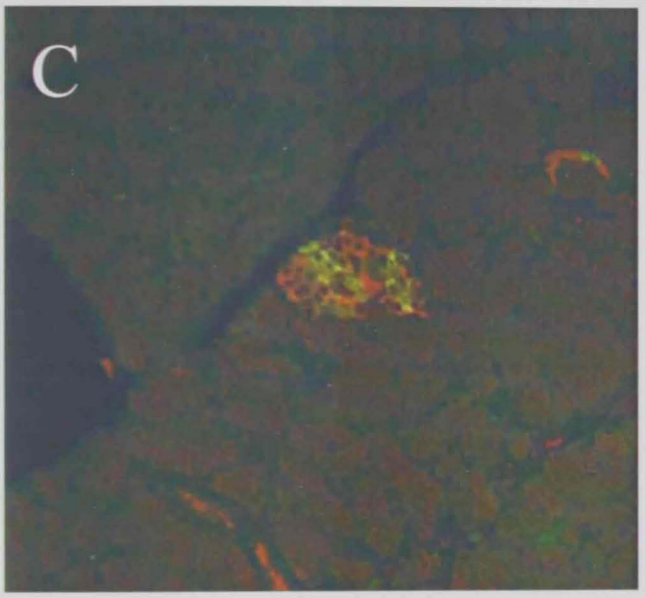




Figure (26) representative micrographs showing somatostatin positive cell (Green) and adiponectin positive cell (Red) in islets of diabetic treated Wister rats.

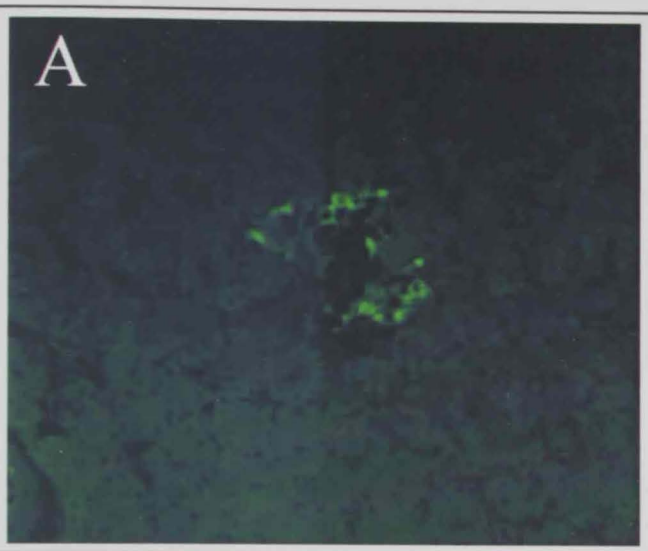
A- Somatostatin (Alone)

B- Adiponectin (Alone)

C- Merged

*Note that some islet cells contain both adiponectin and somatostatin

**Magnification: X 200**





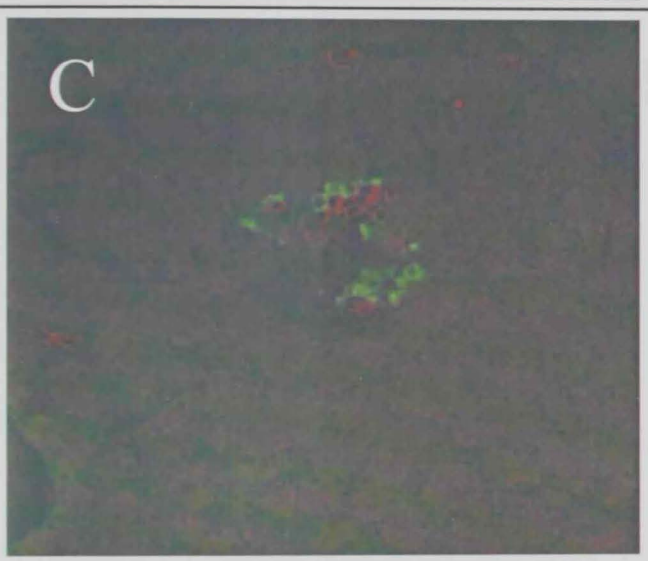



Figure (27) representative micrographs showing somatostatin positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic untreated Wistar rats.

A- Somatostatin (Alone)

B- Adiponectin (Alone)

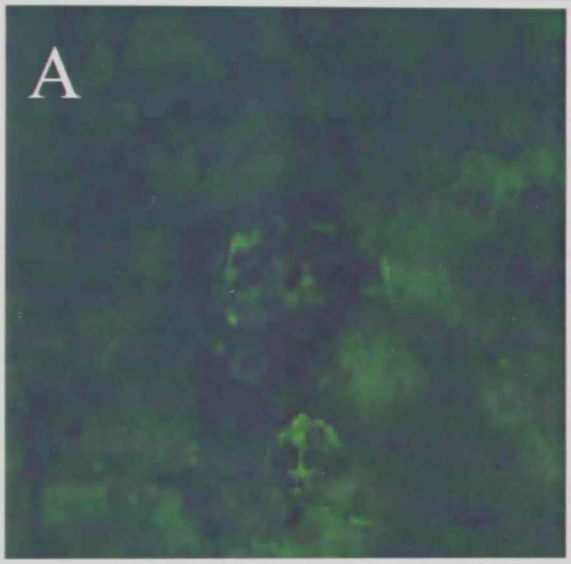
C- Merged

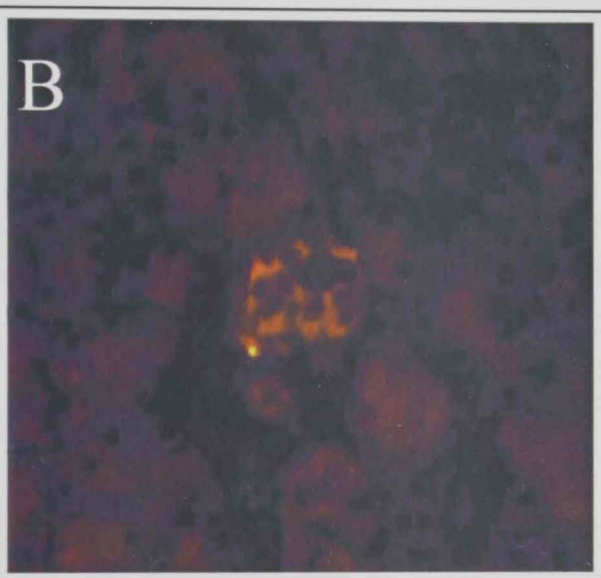
*Not that there is a reduction in the number of adiponectin-positive cells.

**Magnification: X 200**

### III.1.d. Adiponectin and PP

Adiponectin does not co-localize with PP in pancreatic islet cell of GK rats (Figure 28) Treatment of GK rats with adiponectin increased the expression of PP-positive cells in the islet of Langerhans (Figure 29). In normal Wistar rats, administration of adiponectin increased the tissue expression of both adiponectin and PP (Figure 30, 31). The number of adiponectin-positive cells is reduced in untreated diabetic rats (Figure32). Treatment of diabetic Wistar rats did not significantly alter the pattern of distribution of adiponectin (Figure 33) Many cells in the islets of diabetic Wistar rats contain both adiponectin and PP.



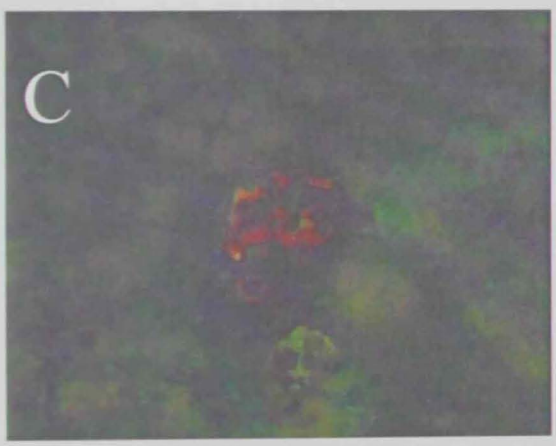



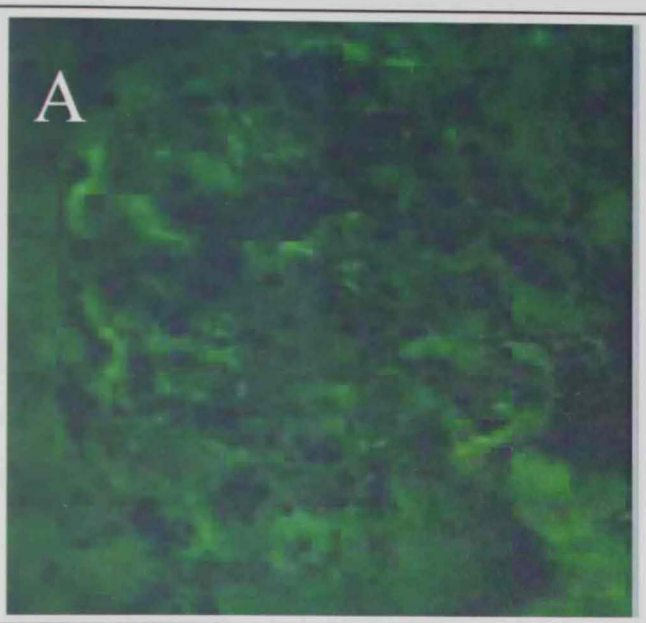

Figure (28) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and Adiponectin positive cell (Red) in pancreas islets of normal GK rats.

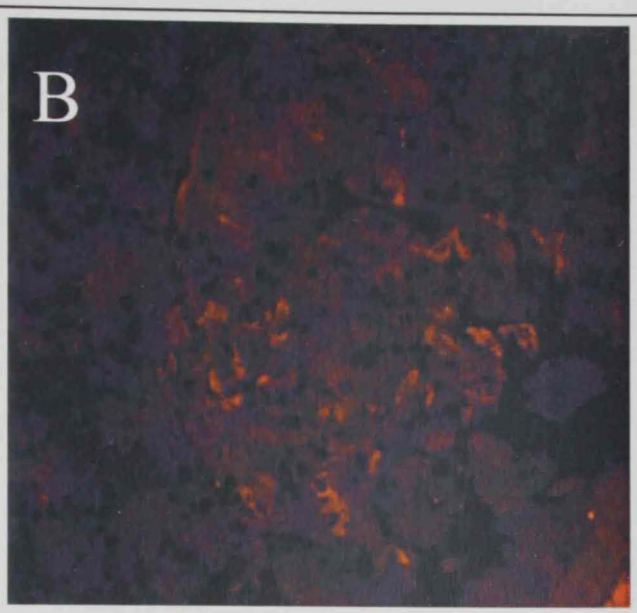
A- PP (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**



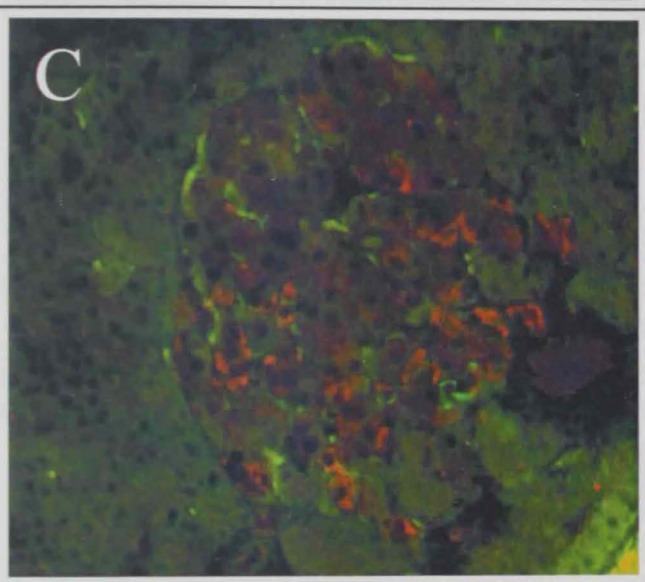



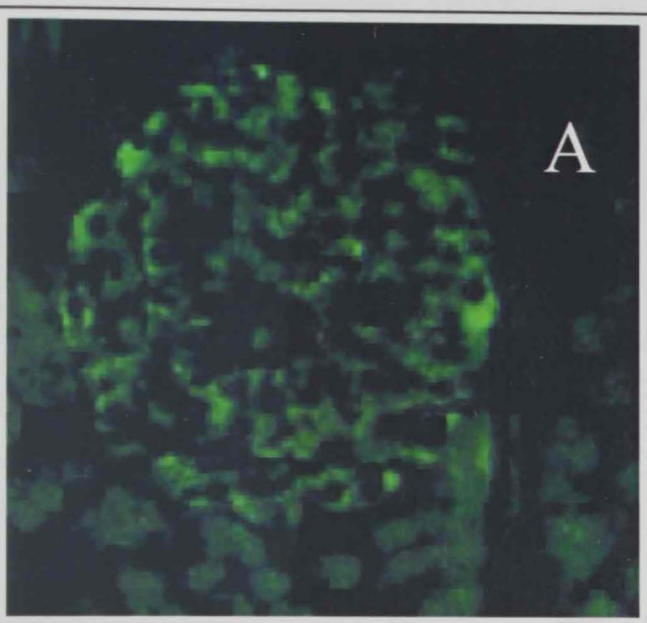

Figure (29) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and Adiponectin positive cell (Red) in pancreas islets of GK treated rats.

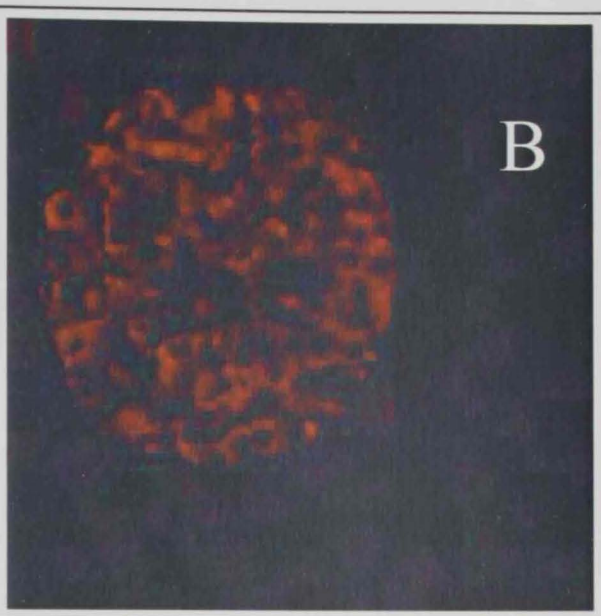
A- PP (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**



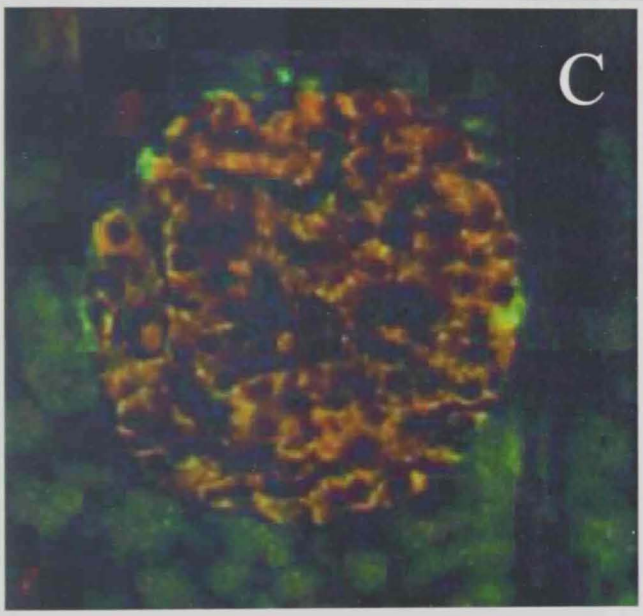




Figure (30) representative micrographs showing PP (Green) and Adiponectin-positive cell (Red) in pancreas islets of normal Wistar rats.

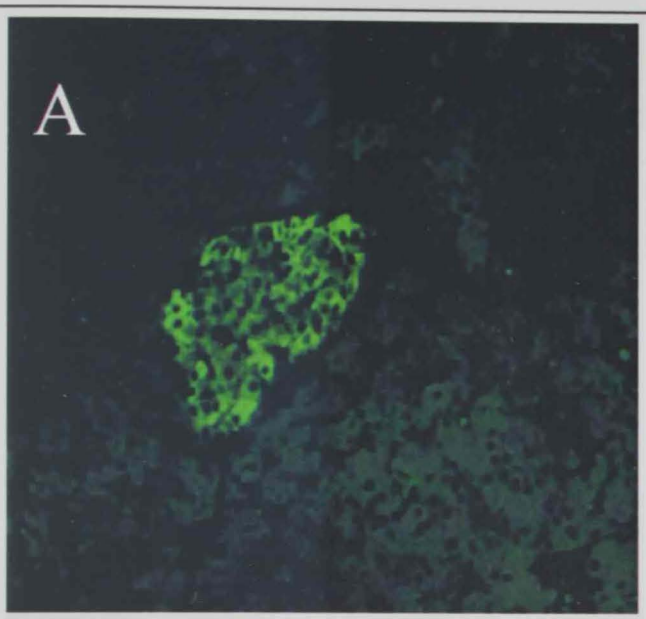
A- PP (Alone)

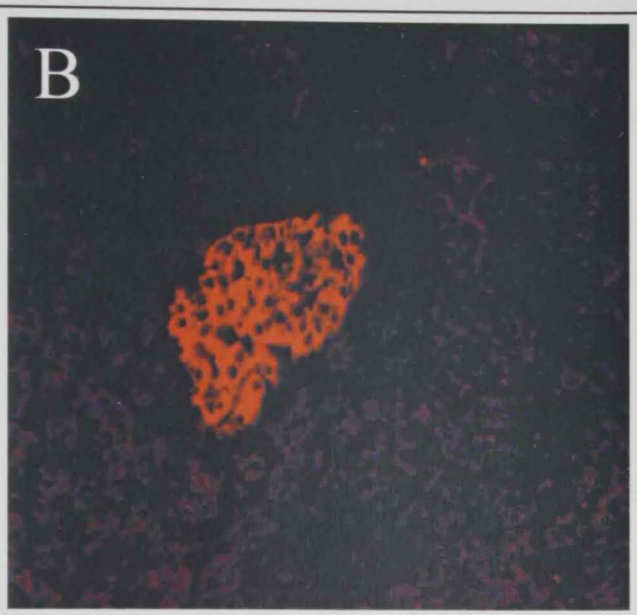
B- Adiponectin (Alone)

C- Merged

*Note that many islet cells contain both PP and adiponectin.

**Magnification: X 200**



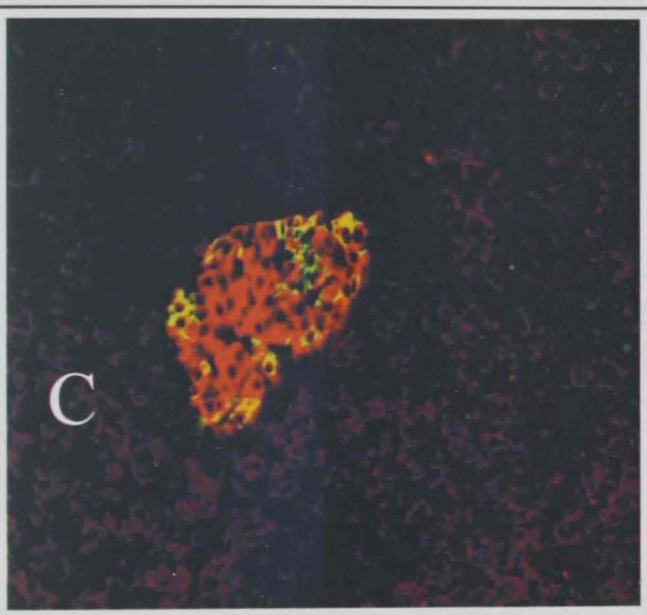




Figure (31) representative micrographs showing PP- positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of normal-treated Wistar rats.

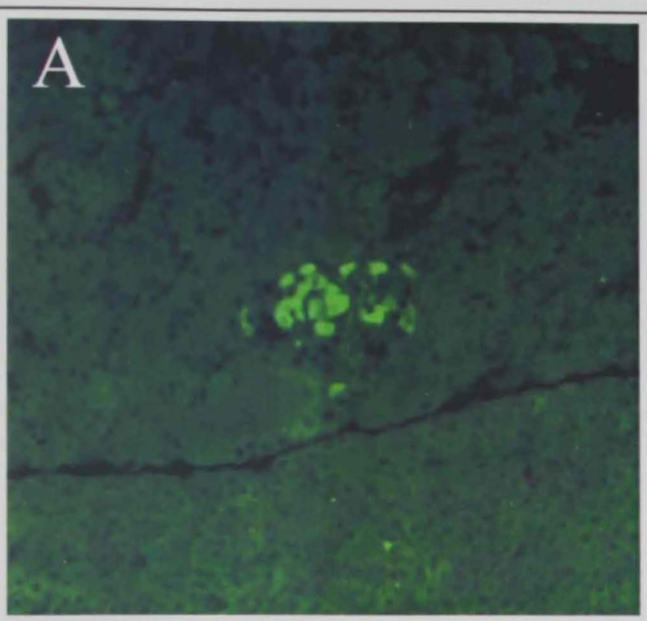
A- PP (Alone)

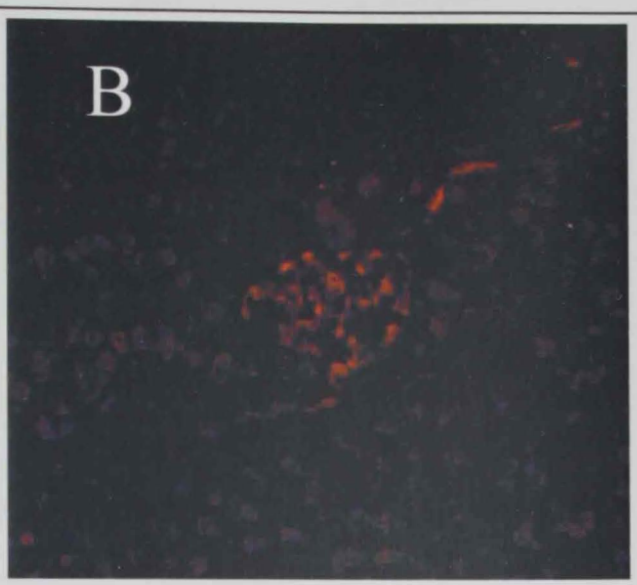
B- Adiponectin (Alone)

C- Merged

*Note that many islet cells contain both PP and adiponectin

**Magnification: X 200**



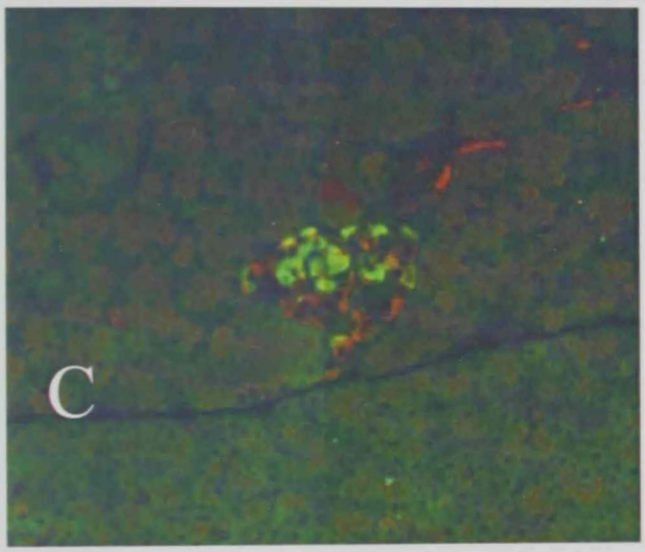



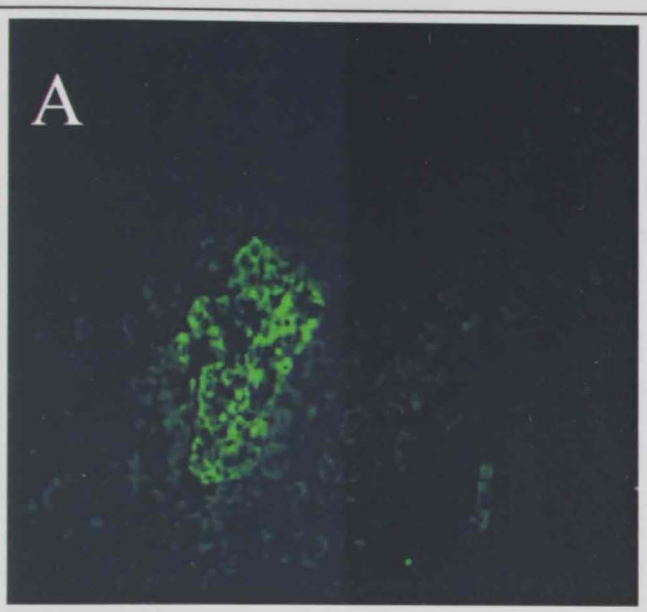

Figure (32) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic untreated Wister rats.

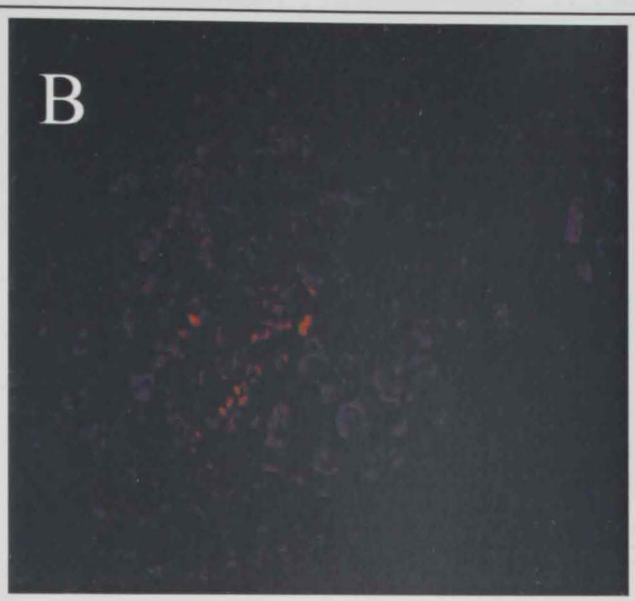
A- PP (Alone)

B- Adiponectin (Alone)

C- Merged

**Magnification: X 200**



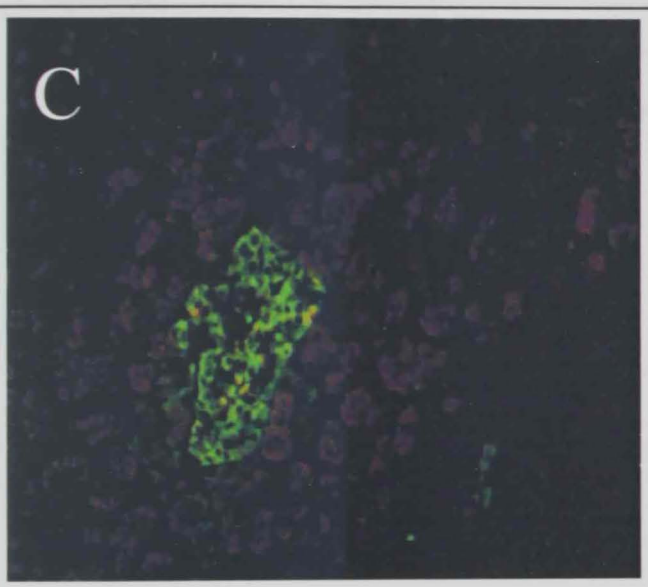




Figure (33) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and adiponectin positive cell (Red) in pancreas islets of diabetic treated Wistar rats

A- PP (Alone)

B- Adiponectin (Alone)

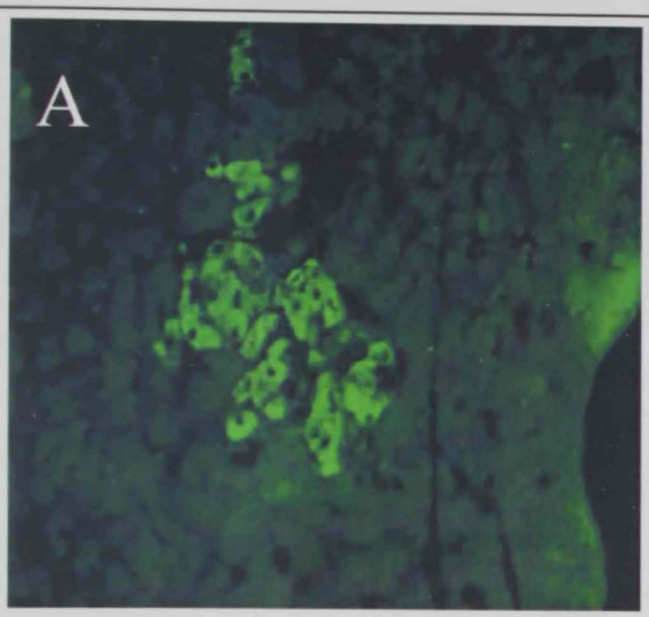
C- Merged

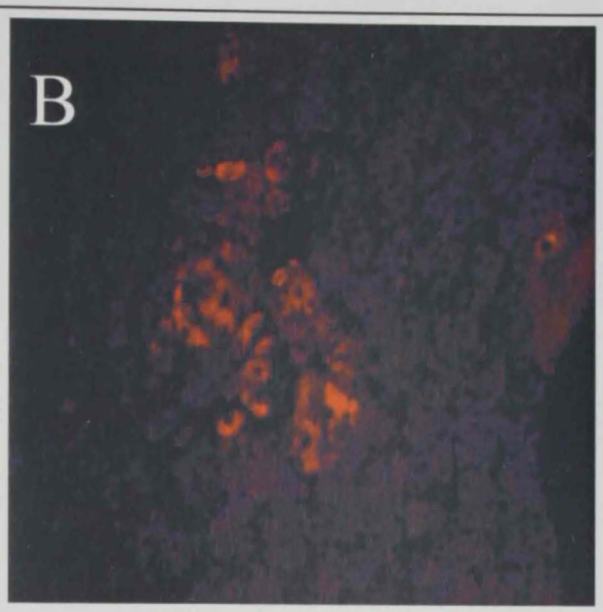
**Magnification: X 200**

## III.2. Immunofluorescence studies of visfatin in pancreatic islets of various animal model of diabetes.

### III.2.a. Visfatin and insulin

Immunofluorescence study shows that many islet cells in the pancreas of GK rats contain visfatin. Many islet cells contain both visfatin as well as insulin (Figure 34). Treatment of GK rats significantly increases the number of visfatin and insulin-containing cells in pancreatic islets (Figure 35). In a similar way, normal and visfatin -treated Wistar rats contain large numbers of visfatin-positive cells (Figure 36). Most of the visfatin-containing cells contain insulin. However, visfatin –positive cells in the periphery of pancreatic islet don not contain insulin. Treatment of normal Wistar rats with visfatin did not affect the pattern of distribution of visfatin (Figure 37). The number of visfatin -immunoreactive cells decreased significantly after the onset of diabetes in Wistar rats (Figure 38)However, treatment of diabetic Wistar with visfatin did not significantly alter the pattern of distribution of visfatin- and insulin-postive cells in the pancreatic islets (Figure 39).



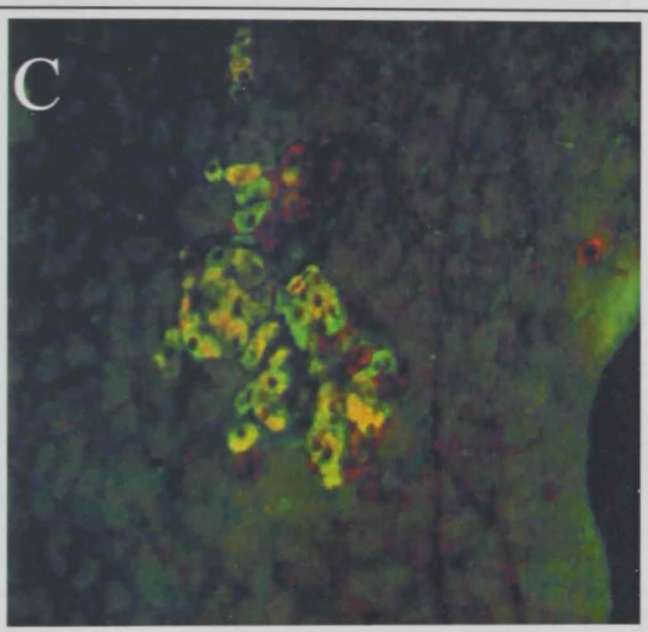




Figure (34) representative micrographs showing insulin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of normal GK rats.

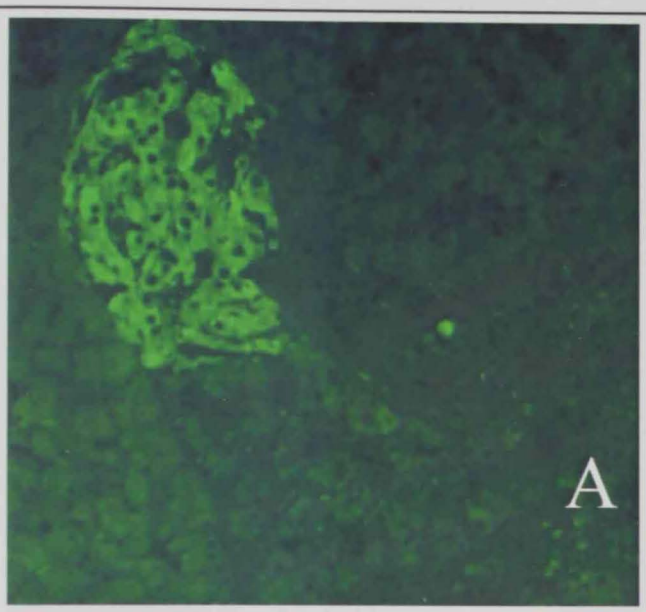
A- Insulin (Alone)

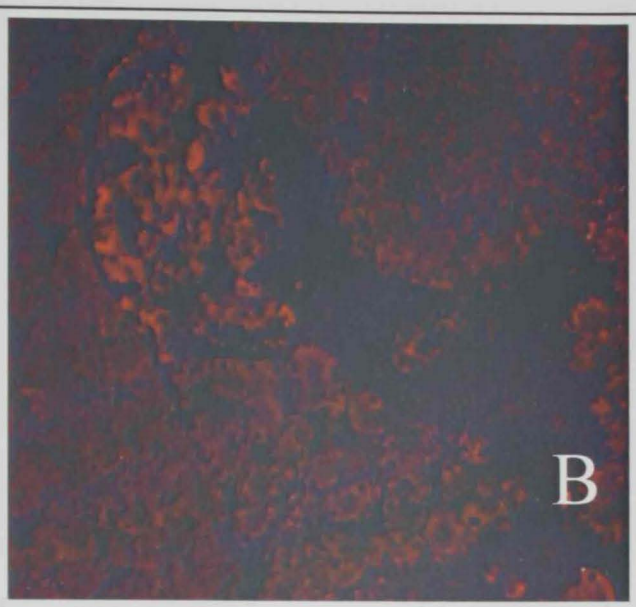
B- Visfatin (Alone)

C- Merged

*Note that there is a high degree of colocalization of insulin and visfatin in islet cells.

**Magnification: X 200**



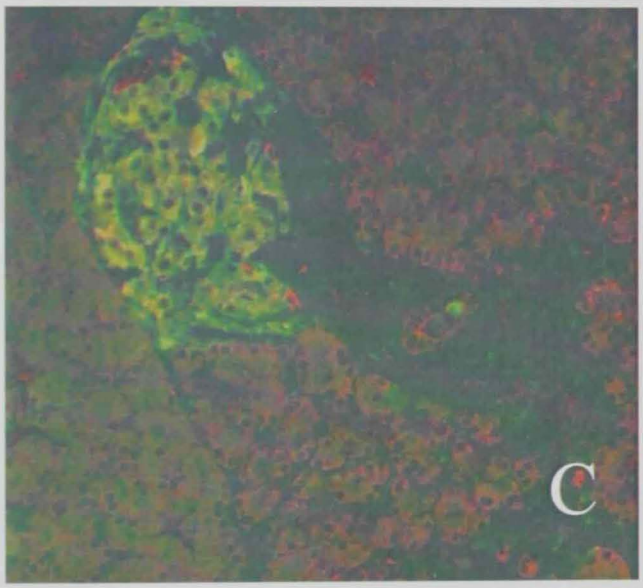




Figure (35) representative micrographs showing insulin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of GK rats treated with visfatin.

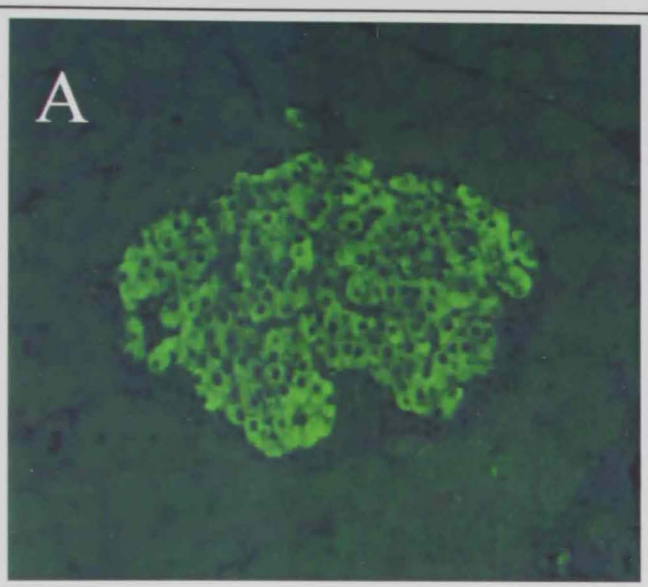
A- Insulin (Alone)

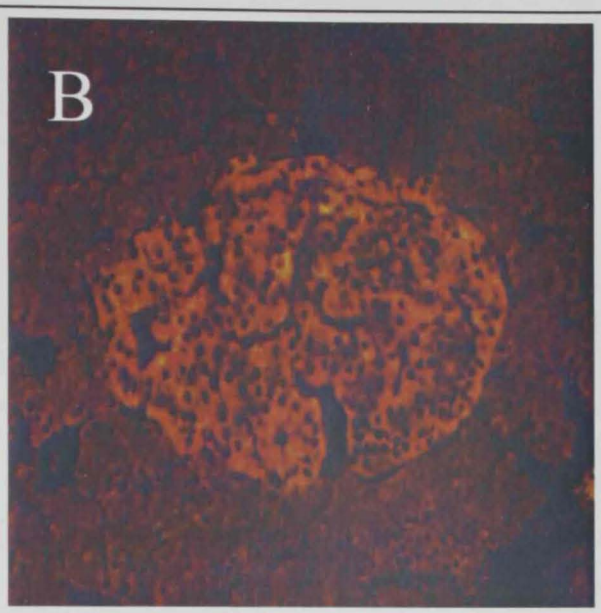
B- Visfatin (Alone)

C- Merged

*Note that the number of islet cells appeared to have increased after visfatin treatment

**Magnification: X 200**



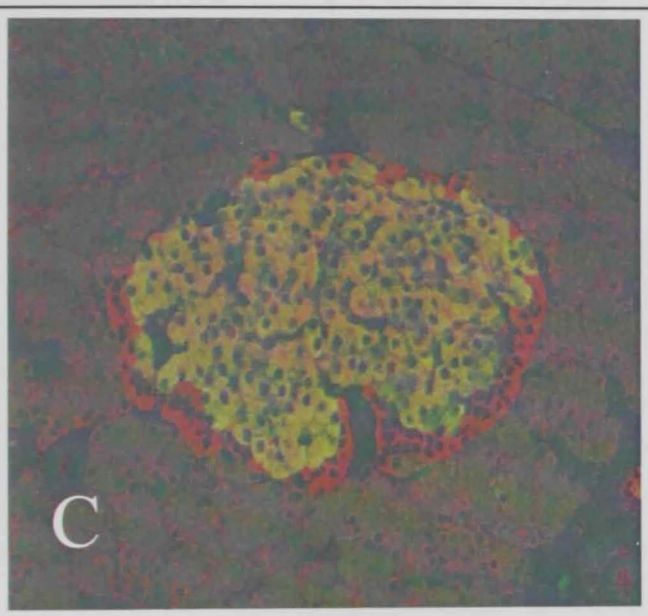




Figure (36) representative micrographs showing Insulin (Green) and Visfatin - positive cell (Red) in pancreas islets of Normal Wistar rats.

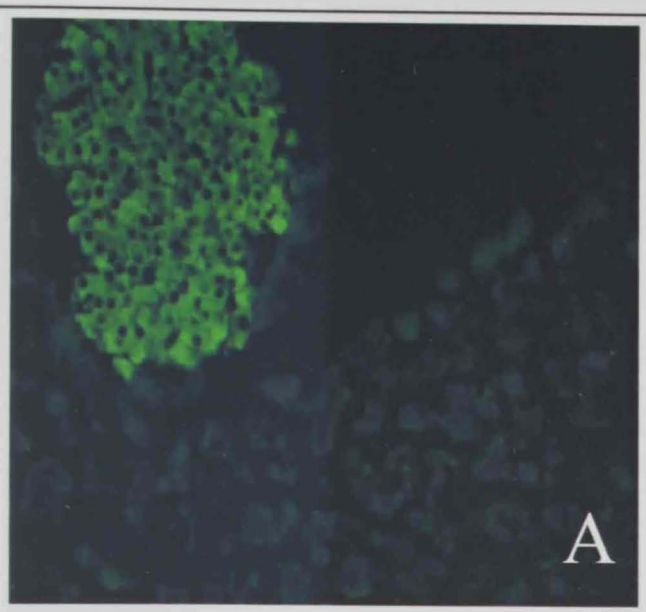
A- Insulin (Alone)

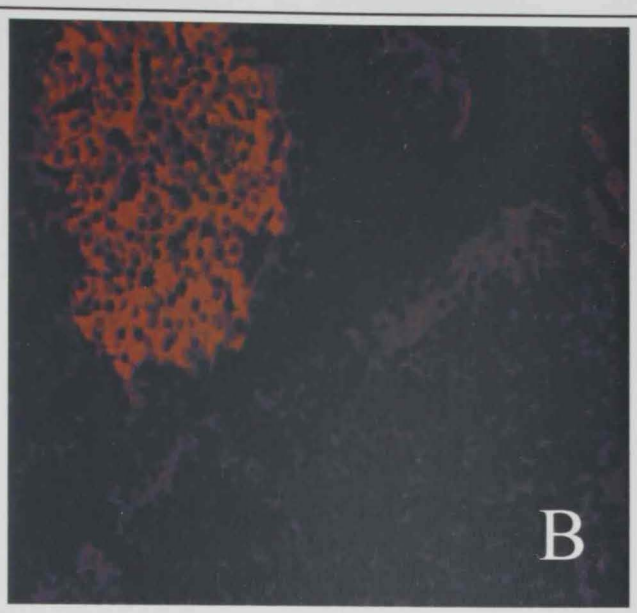
B- Visfatin (Alone)

C- Merged

*Note the high degree of co-localization between insulin and visfatin (yellow)

**Magnification: X 200**



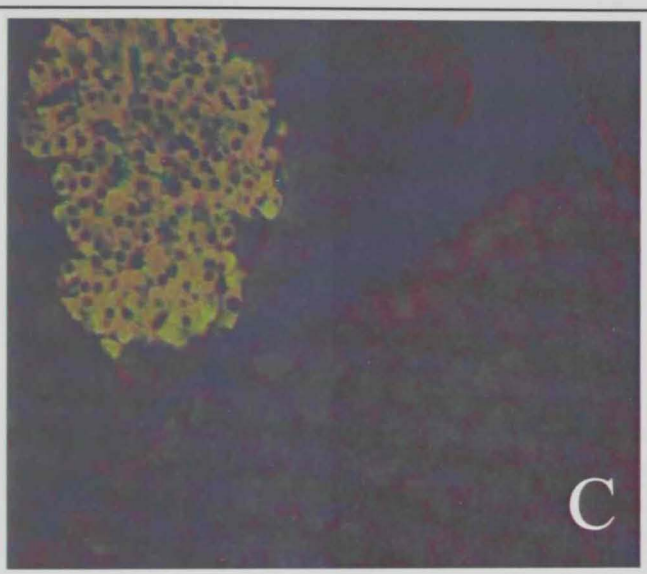




Figure (37) representative micrographs showing insulin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of normal-treated Wistar rats.

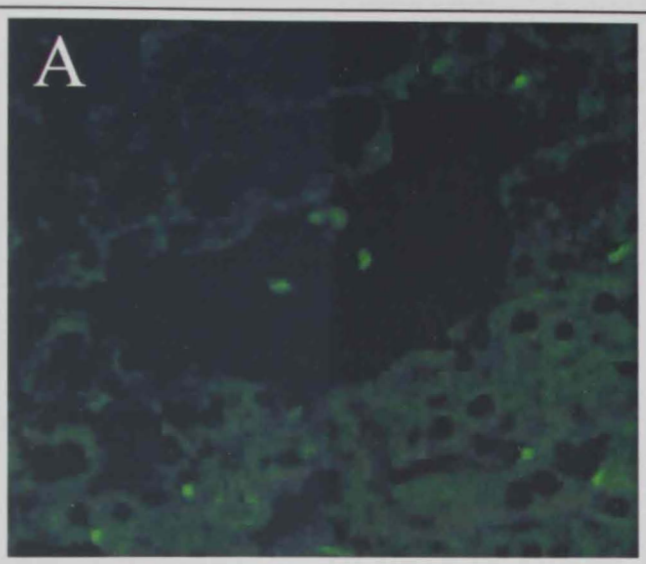
A- Insulin (Alone)

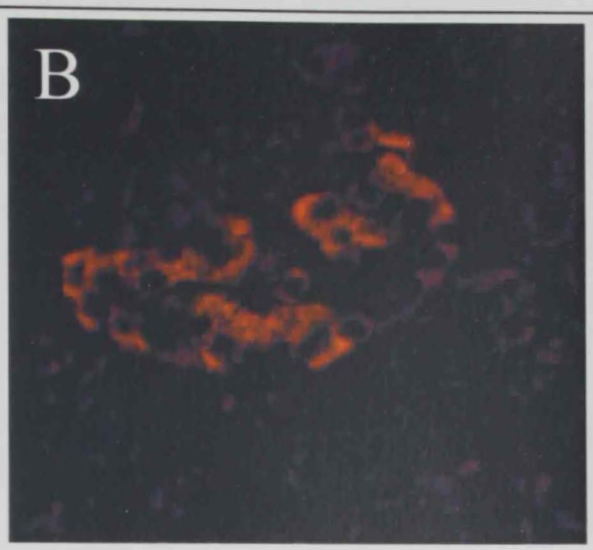
B- Visfatin (Alone)

C- Merged

*Note the high degree of co-localization between insulin and visfatin (yellow)

**Magnification: X 200**



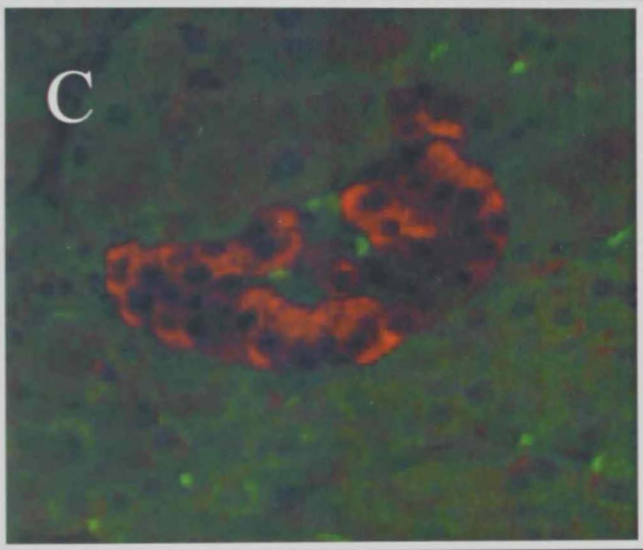




Figure (38) representative micrographs showing insulin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of diabetic un-treated Wistar rats.

A- Insulin (Alone)

B- Visfatin (Alone)

C- Merged

**Magnification: X 200**



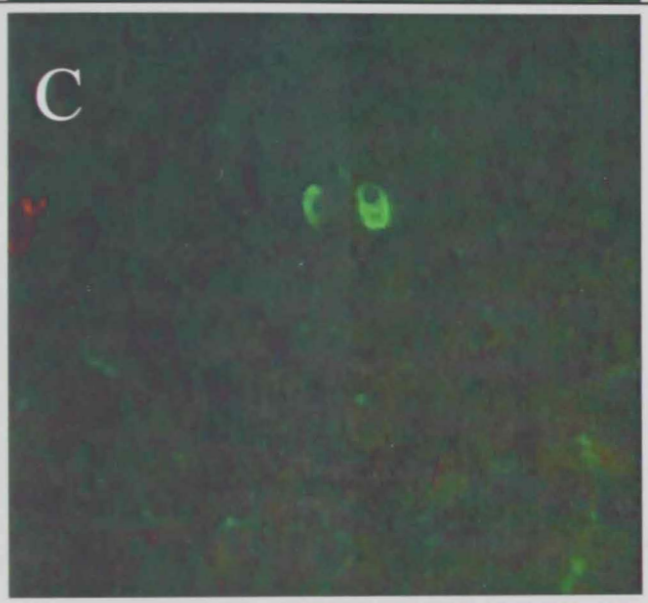



Figure (39) representative micrographs showing insulin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of diabetic treated Wistar rats.

A- Insulin (Alone)

B- Visfatin (Alone)

C- Merged

*Note that visfatin treatement did not prevent the reduction in insulin and visfatin-containging cells

**Magnification: X 200**

### III.2.b. Visfatin and glucagon

The pancreatic islets of GK rats contain large number of glucagon as well as visfatin-positive cells (Figure 40). Visfatin does not co-localize with glucagon in pancreatic islets of GK rats. Treatment of GK rats with visfatin reduced the number of glugacon-immunoreactive cells and the degree of co-localization of visfatin and glucagon (Figure 41). Visfatin is present in glucagon-containg cells in pancreatic islets of normal Wistar rats (Figure 42). The pattern of co-localization of visfatin with glucagon remained the after treatment of normal Wistar rats with visfatin (Figure 43). Treatment of diabetic Wistar rats with visfatin enhanced cellular co-localization between visfatin and glucagon (Figure 44).

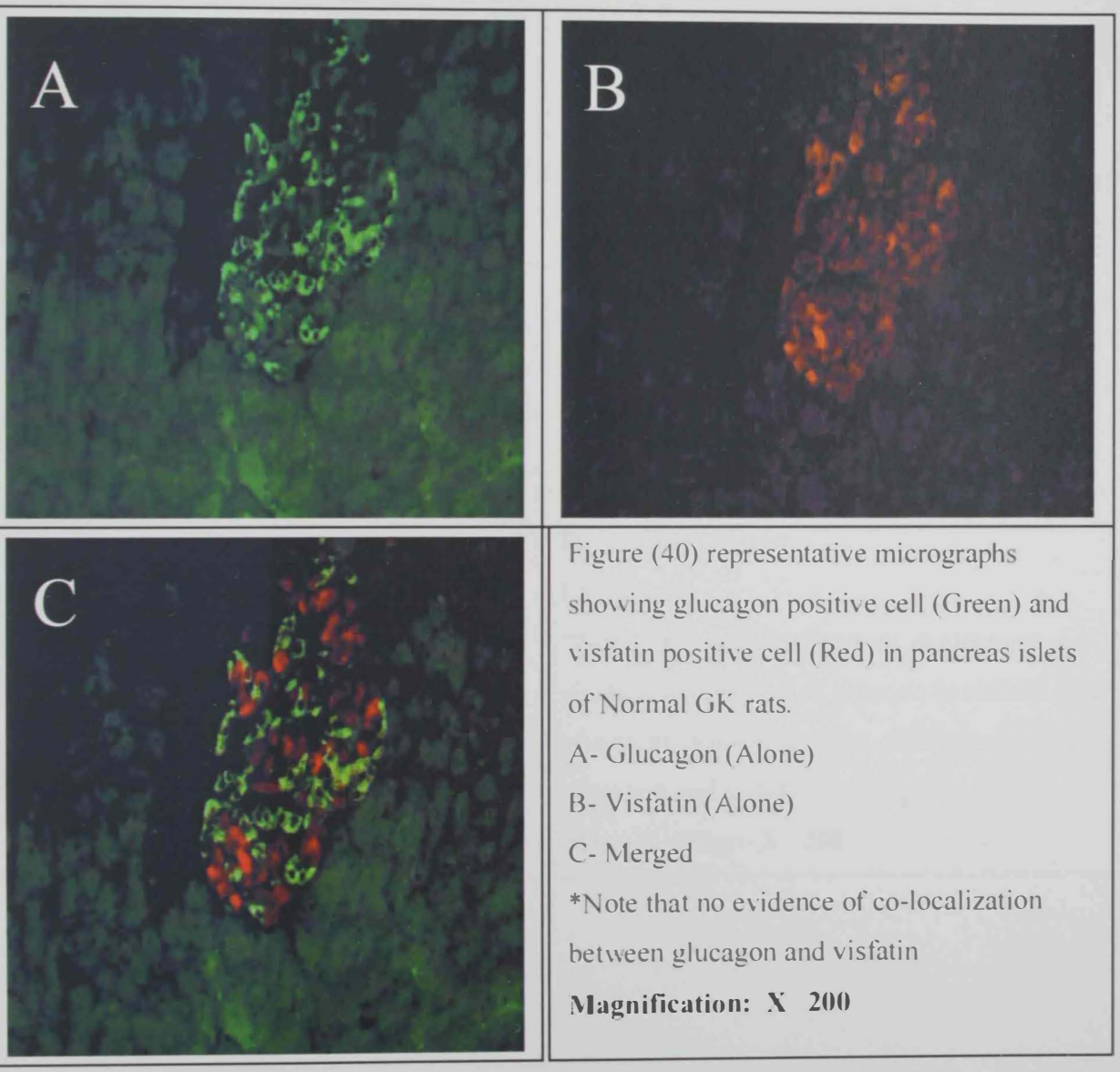



Figure (40) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of Normal GK rats.

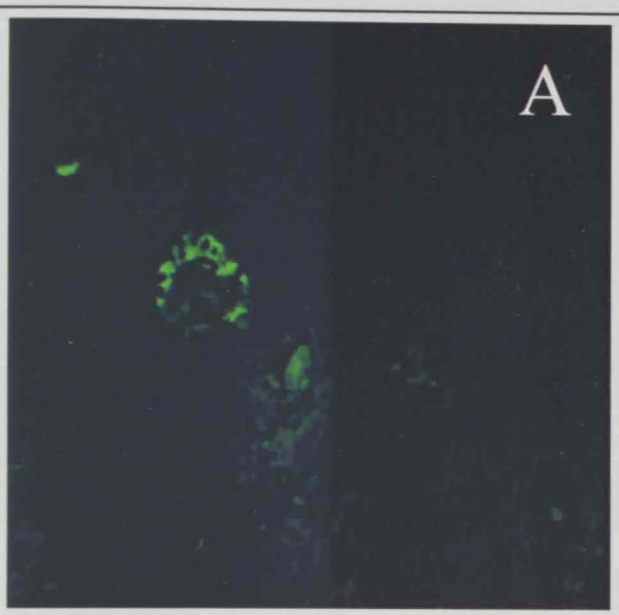
A- Glucagon (Alone)

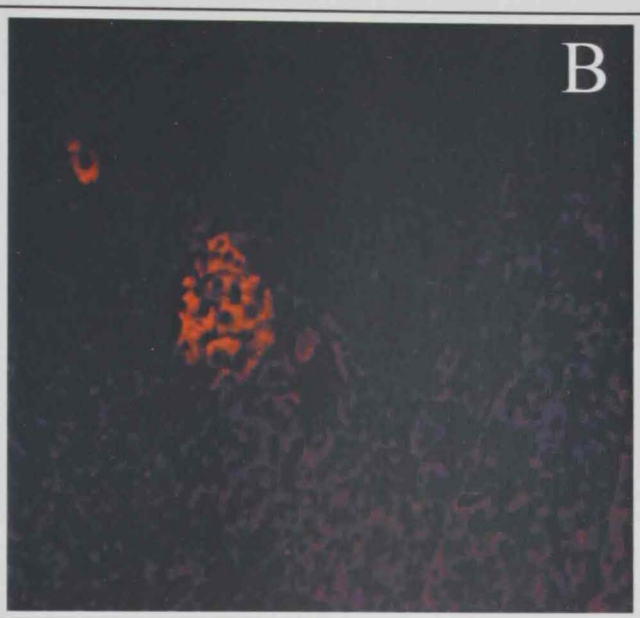
B- Visfatin (Alone)

C- Merged

*Note that no evidence of co-localization between glucagon and visfatin

**Magnification: X 200**



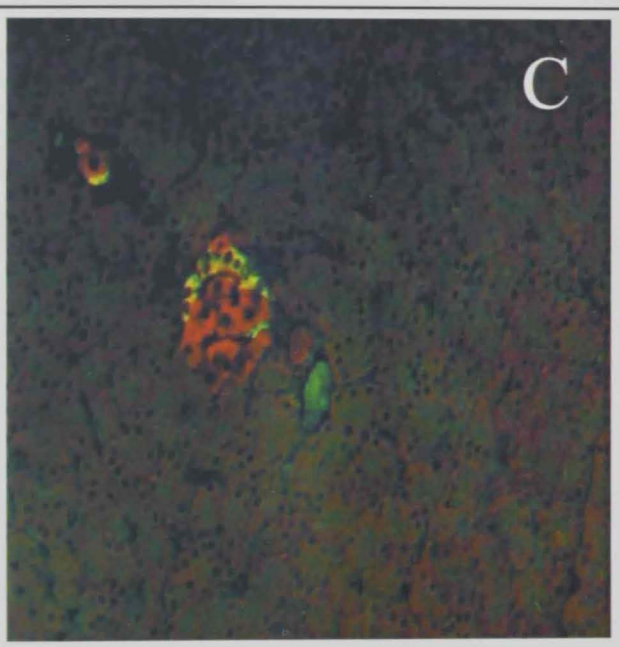




Figure (41) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of treated GK rats.

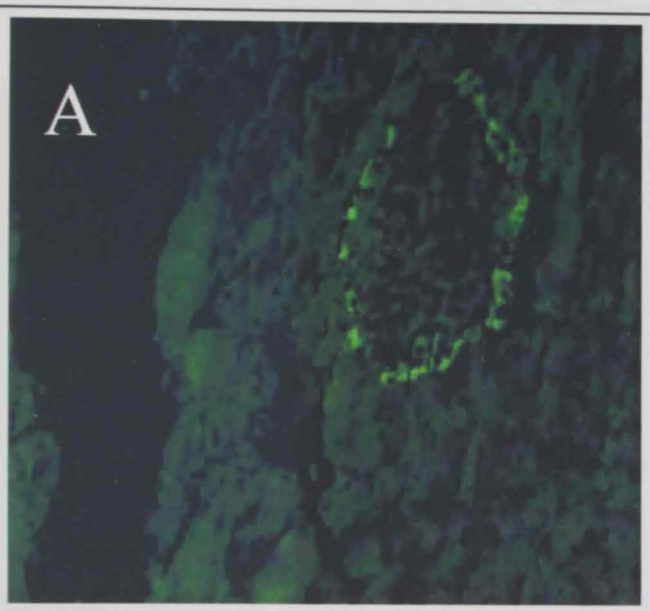
A- Glucagon (Alone)

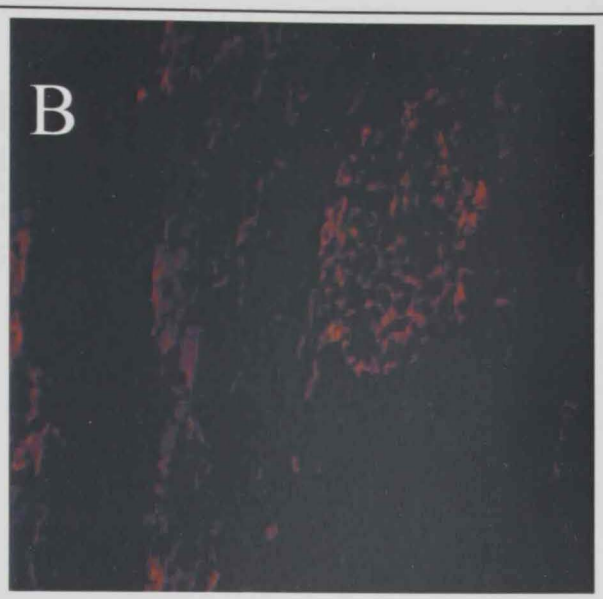
B- Visfatin (Alone)

C- Merged

*Note that a relative decrease in glucagon coupled with a relative increase in visfatin after treatment. Some endocrine cells contain both glucagon and visfatin.

**Magnification: X 200**



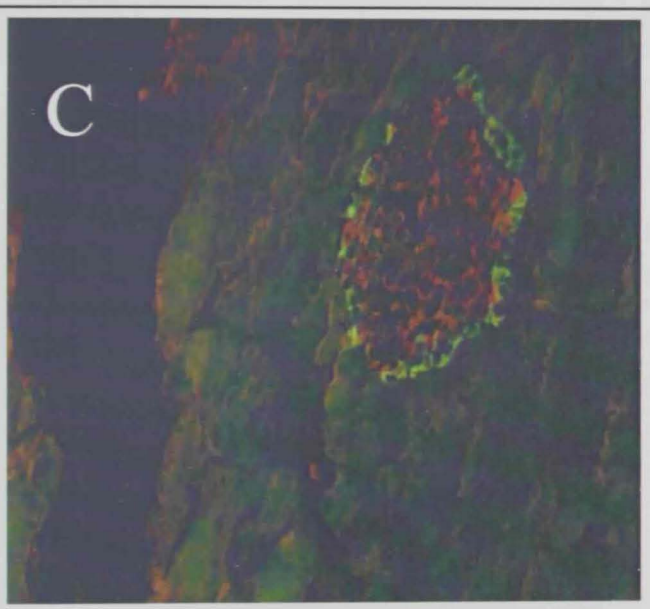




Figure (42) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of normal Wistar rats.

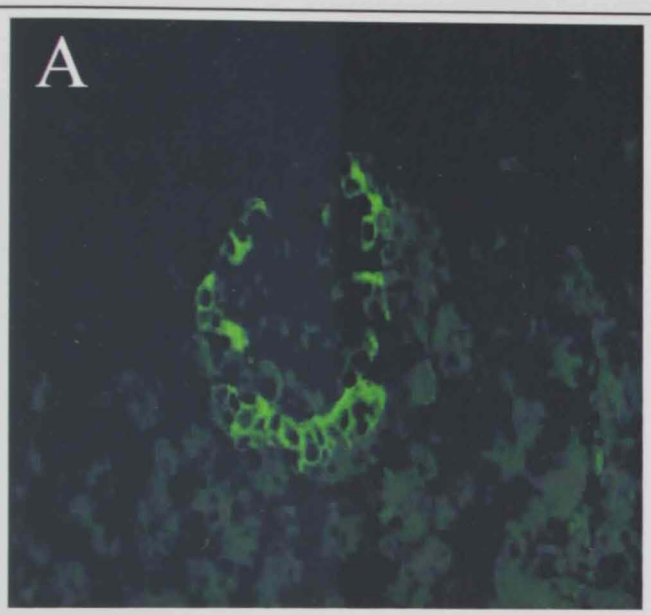
A- Glucagon (Alone)

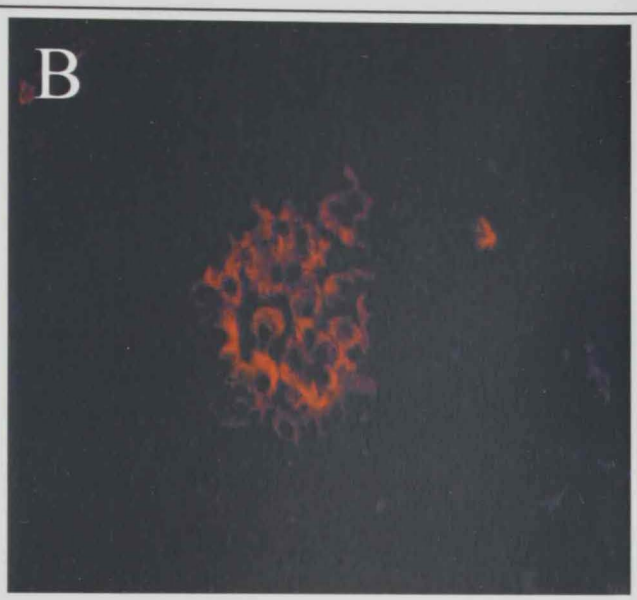
B- Visfatin (Alone)

C- Merged

*Note that some islet cells located in the periphery contain both glucagon and visfatin.

**Magnification: X 200**



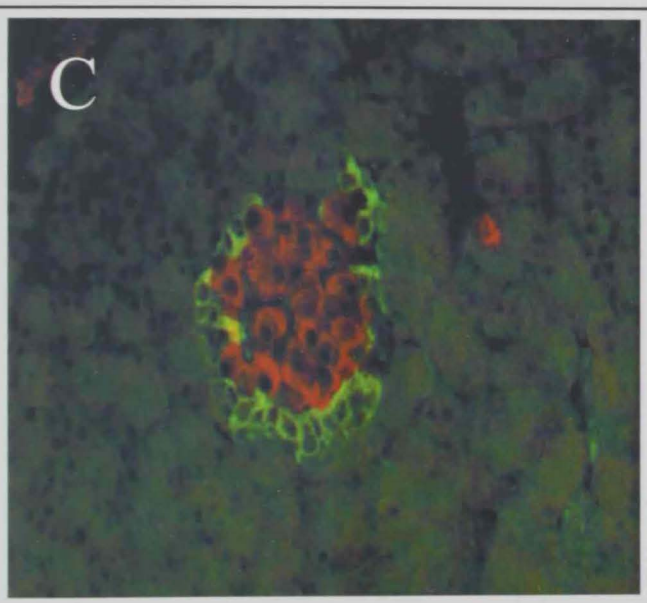




Figure (43) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of normal-treated Wistar rats.

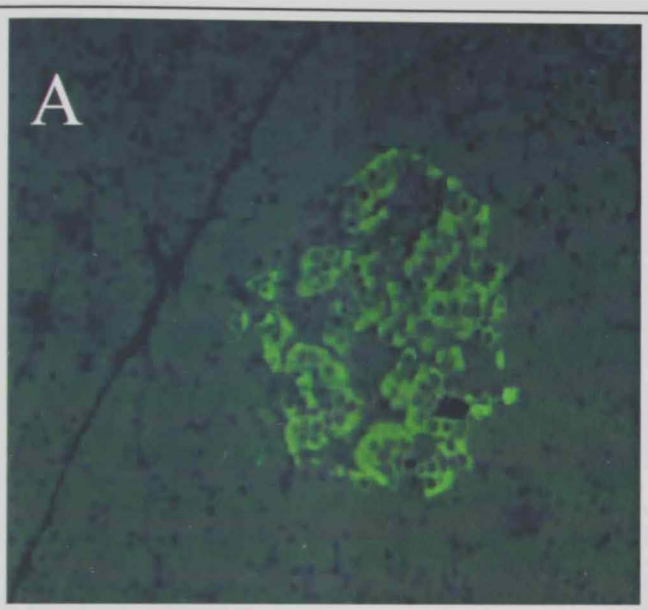
A- Glucagon (Alone)

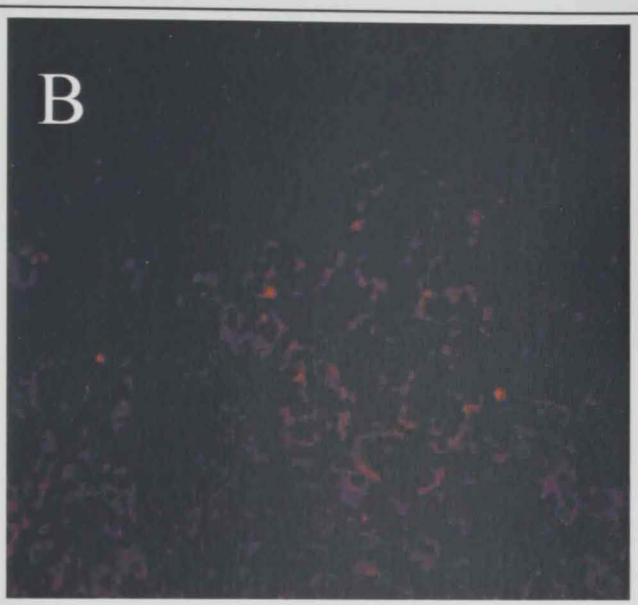
B- Visfatin (Alone)

C- Merged

*Note that some islet cells located in the periphery contain both glucagon and visfatin.

**Magnification: X 200**



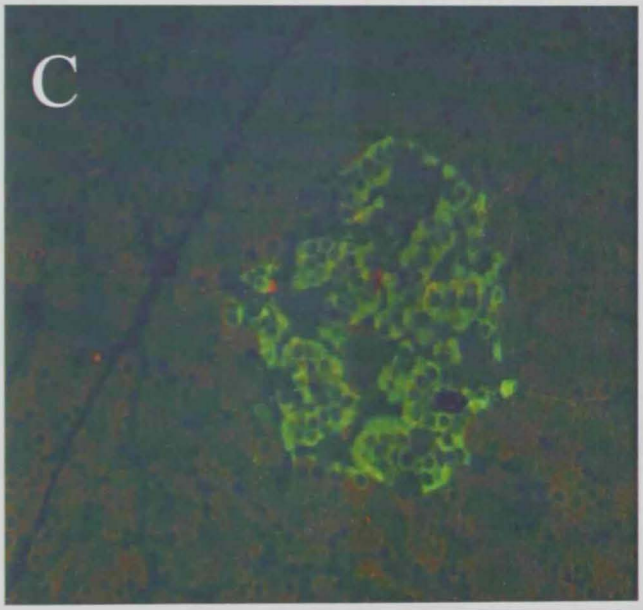



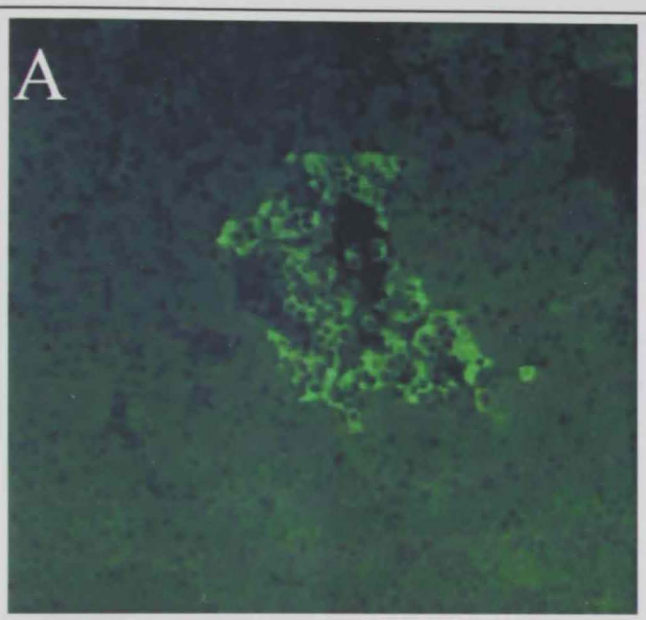

Figure (44) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of diabetic treated Wistar rats.

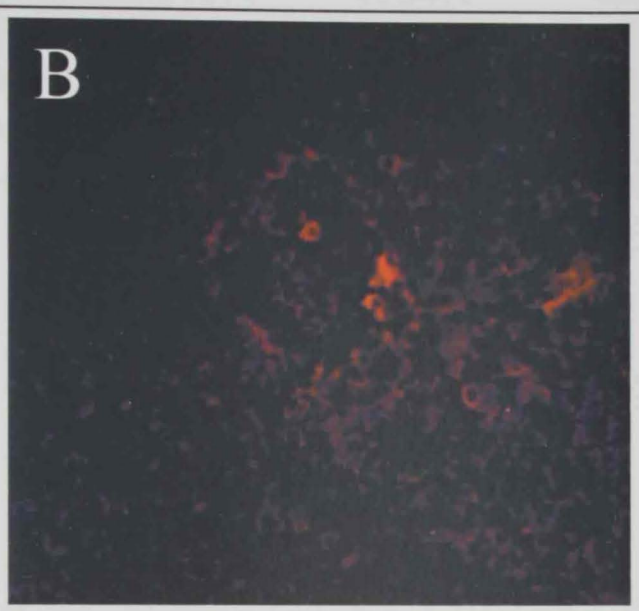
A- Glucagon (Alone)

B- Visfatin (Alone)

C- Merged

**Magnification: X 200**



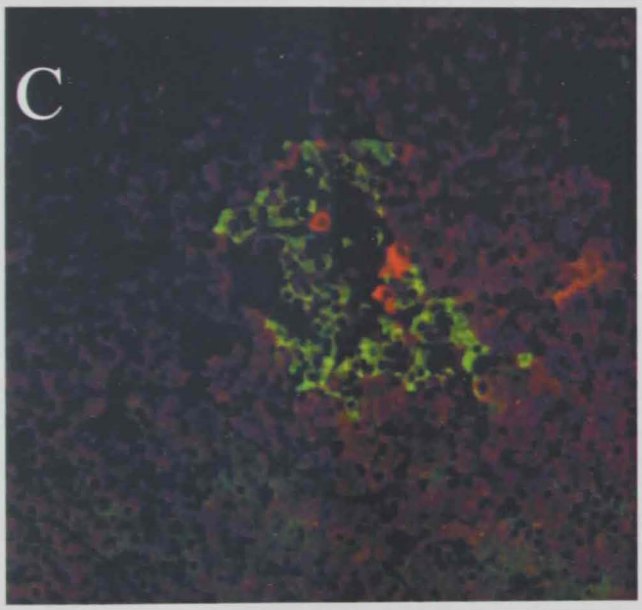




Figure (45) representative micrographs showing glucagon positive cell (Green) and visfatin positive cell (Red) in pancreas islets of diabetic un-treated Wistar rats.

A- Glucagon (Alone)

B- Visfatin (Alone)

C- Merged

*Note that the number of visfatin -positive cells is reduced in diabetes

**Magnification: X 200**

### III.2.c. Visfatin and somatostatin

Visfatin does not co-localize with somatostatin in pancreatic islet cell of GK rats (Figure 46). Treatment of GK rats with visfatin did not alter the pattern of distribution of visfatin and somatostatin in the islet of Langerhans (Figure 47). Normal and treated Wistar rats have a similar pattern of visfatin- somatostatin relation (Figure 48, 49). Treatment of diabetic Wistar rats did not significantly restored the expression of visfatin and reduced that of somatostatin. Some cells in the islets of diabetic Wistar rats contain both visfatin and somatostatin(Figure 50,51).

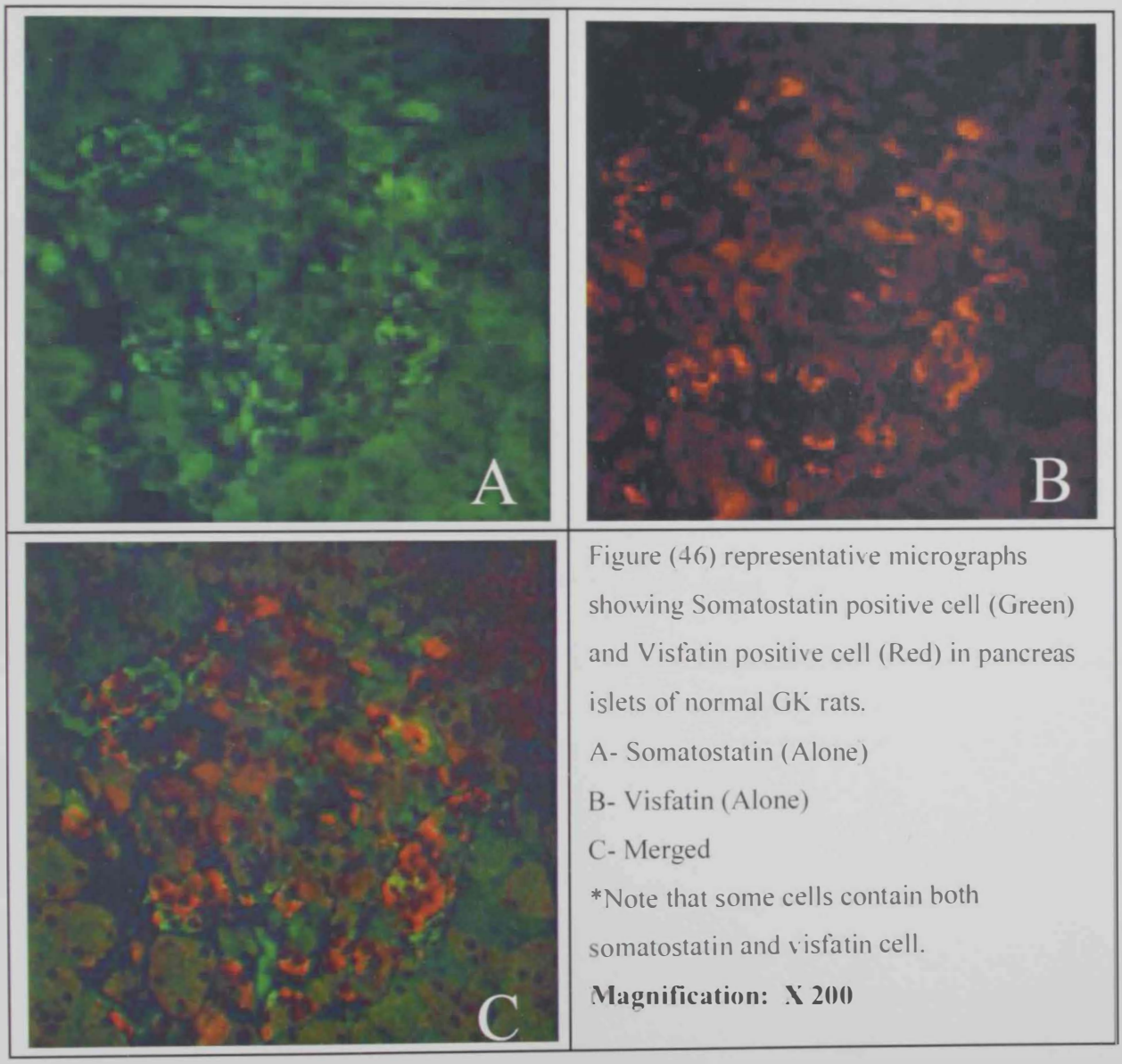



Figure (46) representative micrographs showing Somatostatin positive cell (Green) and Visfatin positive cell (Red) in pancreas islets of normal GK rats.

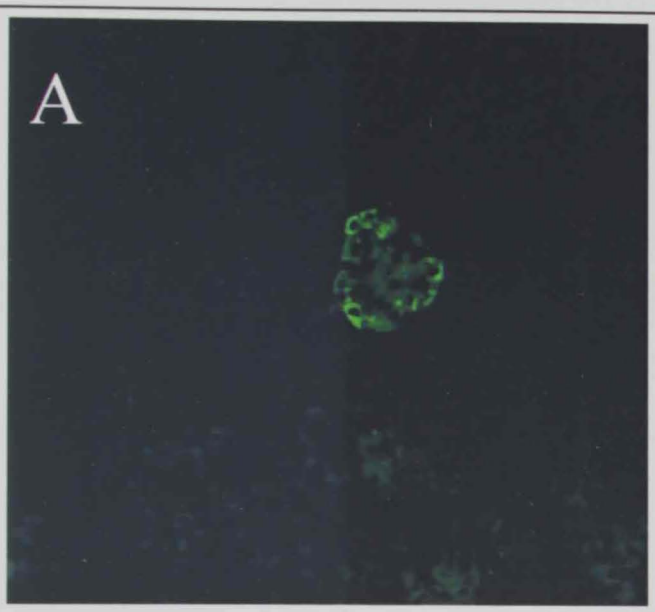
A- Somatostatin (Alone)

B- Visfatin (Alone)

C- Merged

*Note that some cells contain both somatostatin and visfatin cell.

**Magnification: X 200**





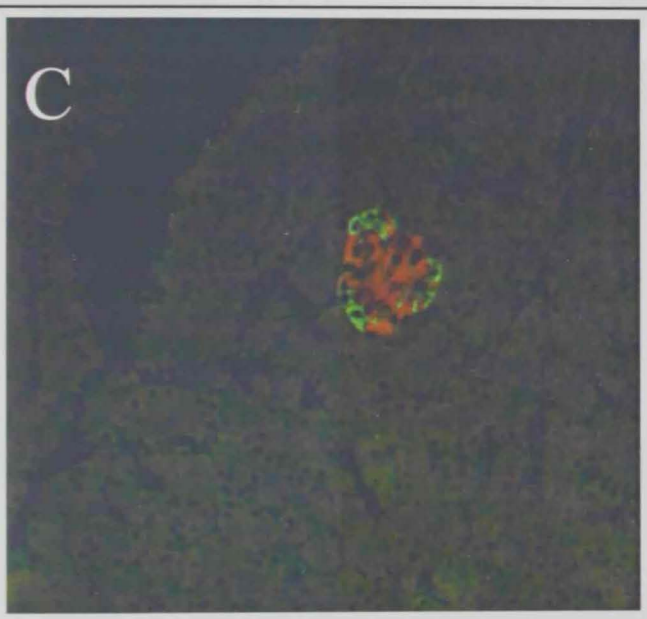



Figure (47) representative micrographs showing somatostatin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of treated GK rats.

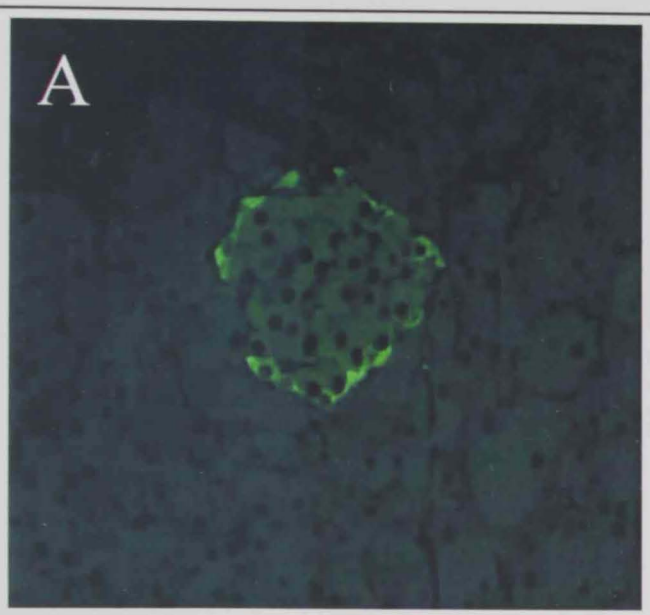
A- Somatostatin (Alone)

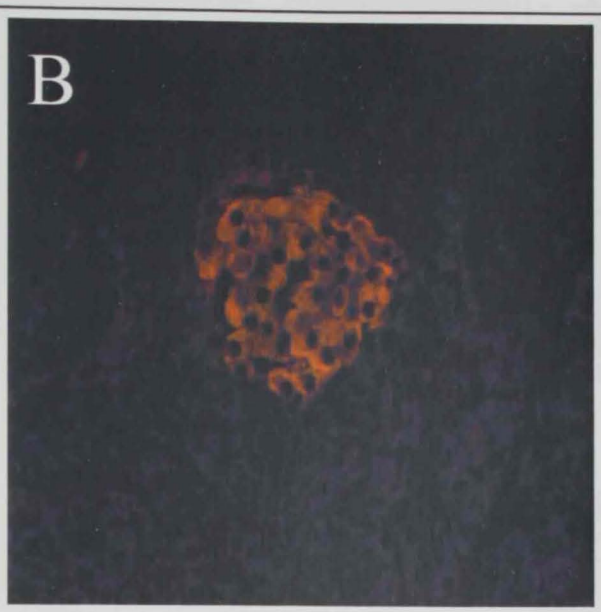
B- Visfatin (Alone)

C- Merged

Some cells contain both somatostatin and visfatin.

**Magnification: X 200**



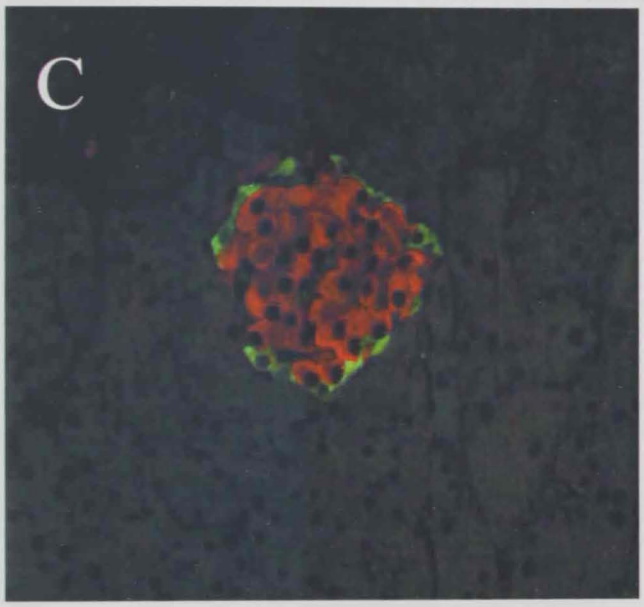




Figure (48) representative micrographs showing somatostatin- (Green) and Visfatin-positive cell (Red) in pancreas islets of normal Wistar rats.

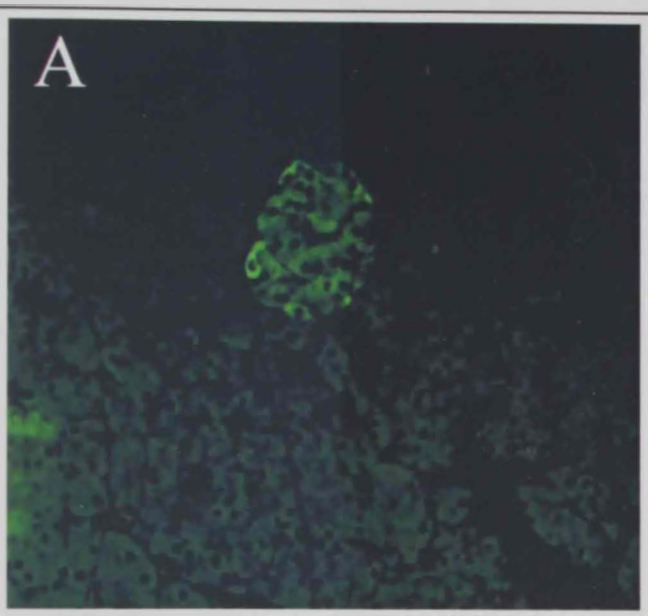
A- Somatostatin (Alone)

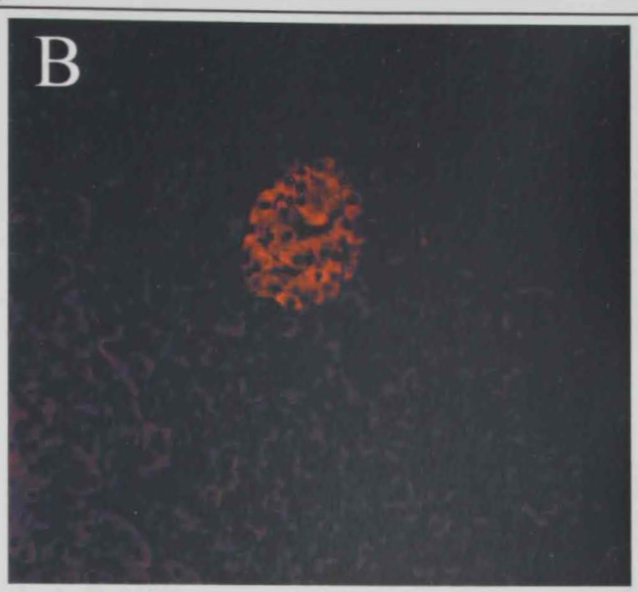
B- Visfatin (Alone)

C- Merged

Note that no co-localization between visfatin and somatostatin.

**Magnification: X 200**



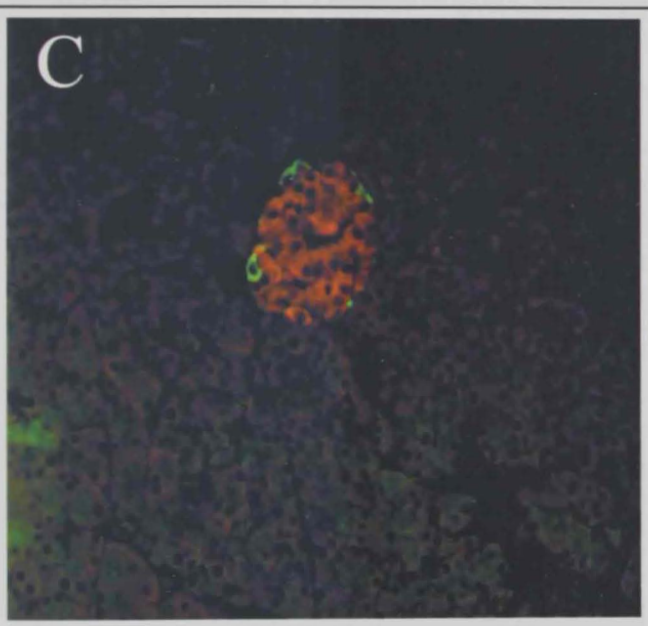




Figure (49) representative micrographs showing somatostatin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of normal-treated Wistar rats.

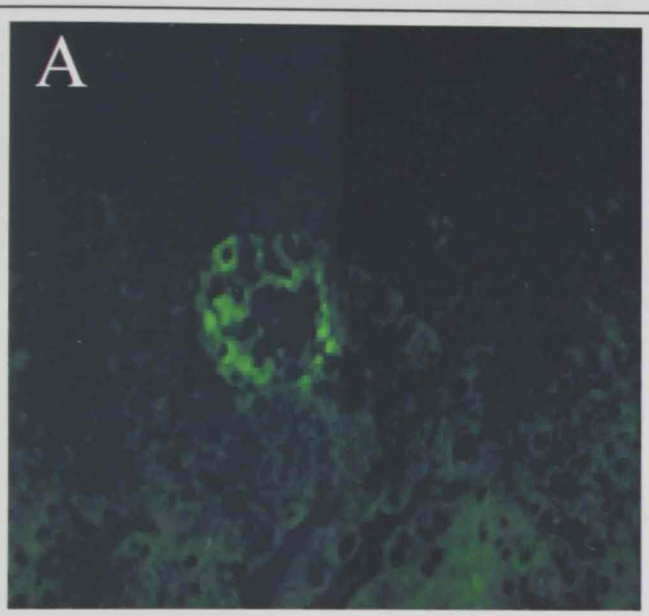
A- Somatostatin (Alone)

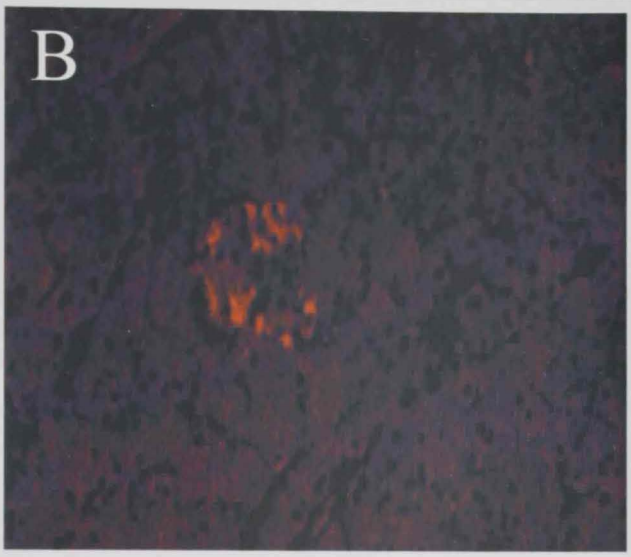
B- Visfatin (Alone)

C- Merged

* Note that no co-localization between visfatin and somatostatin.

**Magnification: X 200**



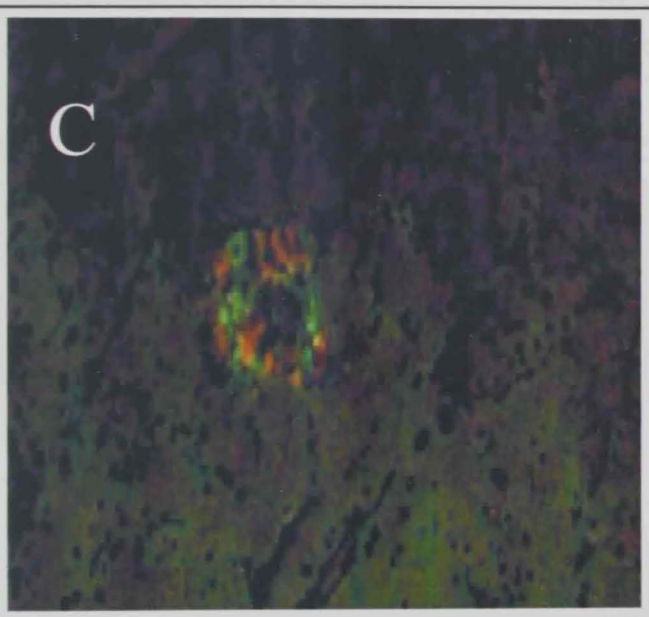



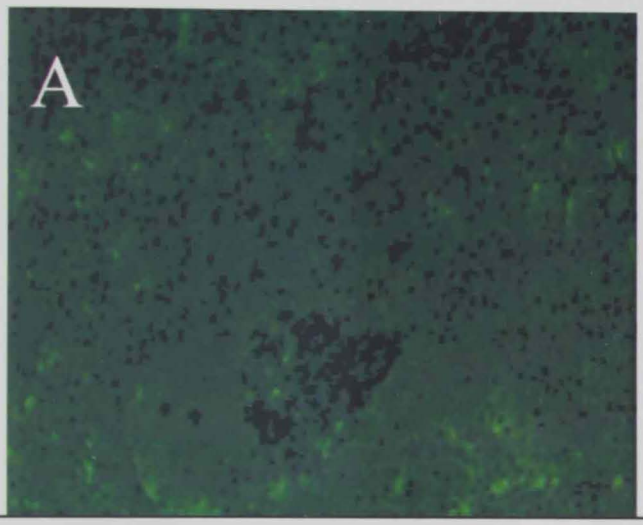

Figure (50) representative micrographs showing somatostatin positive cell (Green) and Visfatin positive cell (Red) in pancreas islets of diabetic un-treated Wistar rats.

A- Somatostatin (Alone)

B- Visfatin (Alone)

C- Merged

**Magnification: X 200**





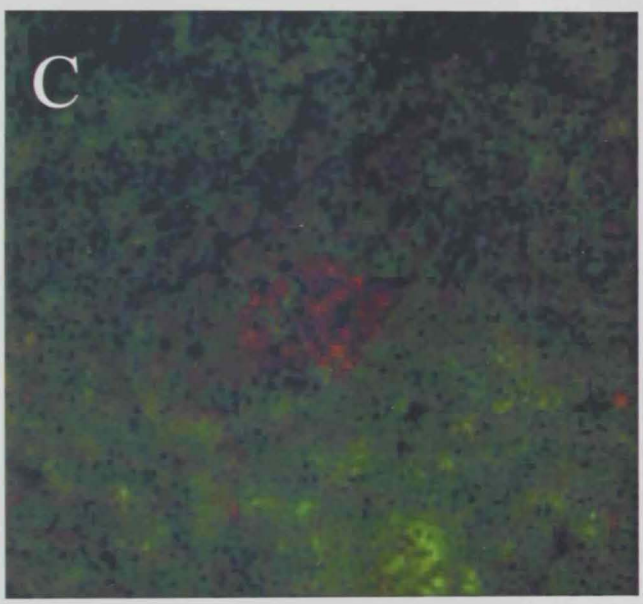



Figure (51) representative micrographs showing somatostatin positive cell (Green) and visfatin positive cell (Red) in pancreas islets of diabetic treated Wistar rats.

A- Somatostatin (Alone)

B- Visfatin (Alone)

C- Merged

*Note that treatment with visfatin reduced the number of somatostatin-positive cells

**Magnification: X 200**

### III.2.d. Visfatin and PP

The islets of GK rats contain large numbers of PP cells. No evidence of co-localization between visfatin and PP in normal untreated GK rats (Figure 52). However, visfatin co-localized with PP in pancreatic islet cell of GK rats treated with visfatin (Figure 53). In normal Wistar rats, administration of visfatin decreased the tissue expression of visfatin. Some cells in islet periphery contain both visfatin and PP (Figure 54, 55).

The number of visfatin-positive cells is reduced in untreated diabetic rats (Figure 56). However, treatment of diabetic Wistar rats, increased tissue expression of both visfatin and PP. Many cells in the islets of diabetic Wistar rats contain both visfatin and PP (Figure 57).

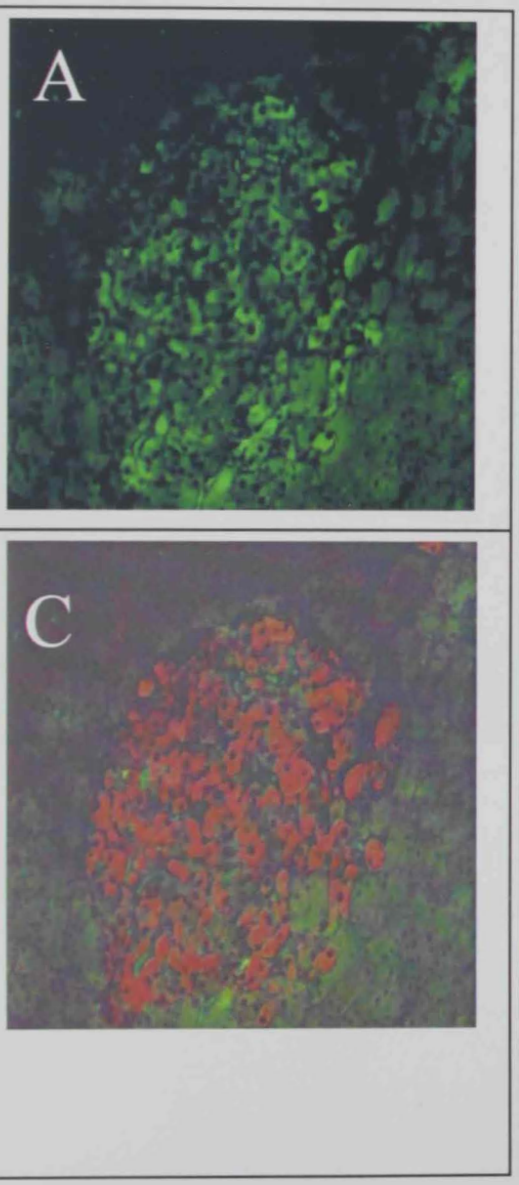



Figure (52) representative micrographs showing pancreatic polypeptide positive cell (Green) and visfatin positive cell (Red) in pancreas islets of Normal GK rats.

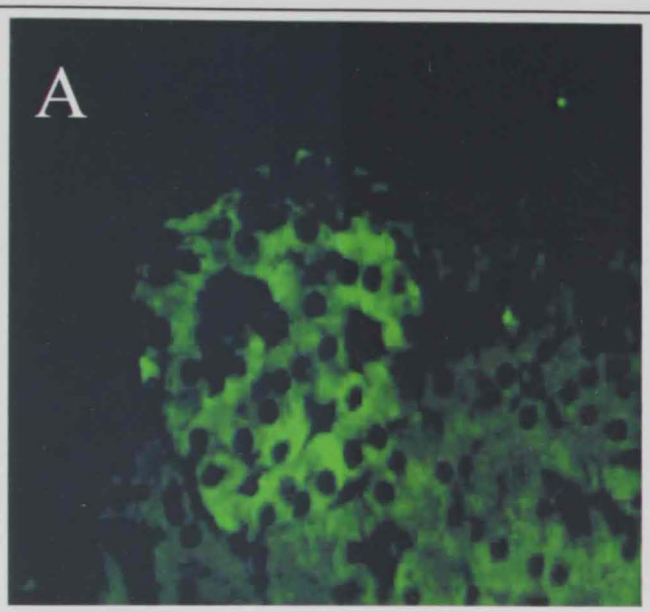
A- PP (Alone)

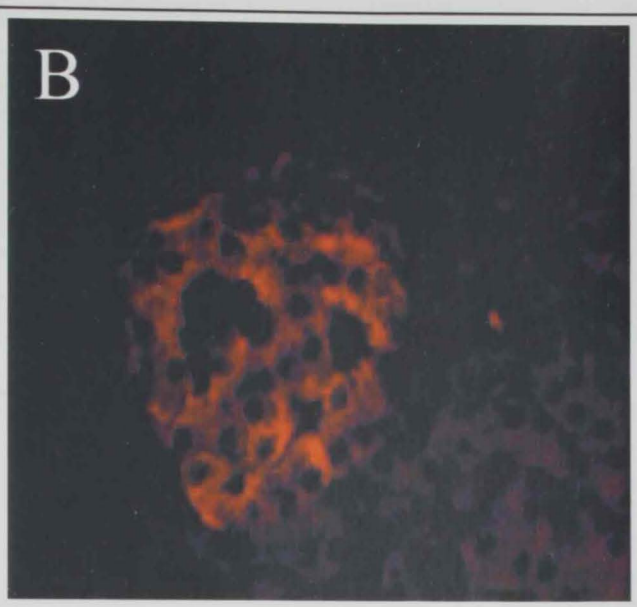
B- Visfatin (Alone)

C- Merged

Note that the islets of normal GK rats contain large quantitities of visfatin and PP with no evidence of co-localization.

**Magnification: X 200**



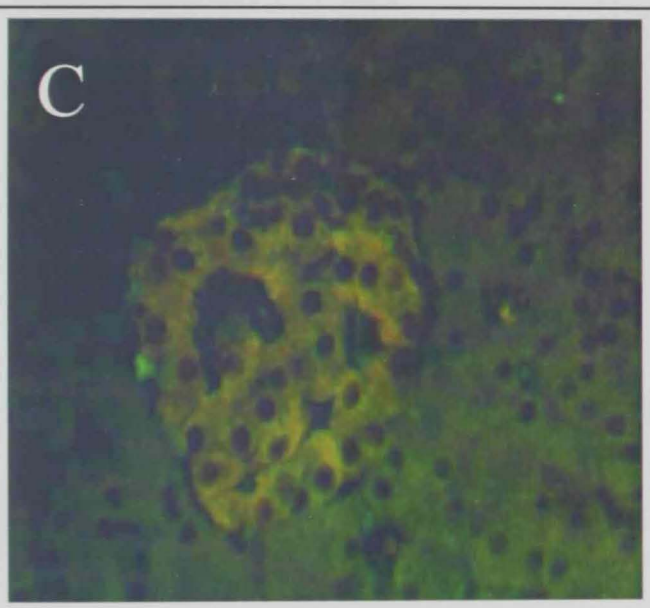




Figure (53) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and Visfatin positive cell (Red) in pancreas islets of GK- rats treated with visfatin.

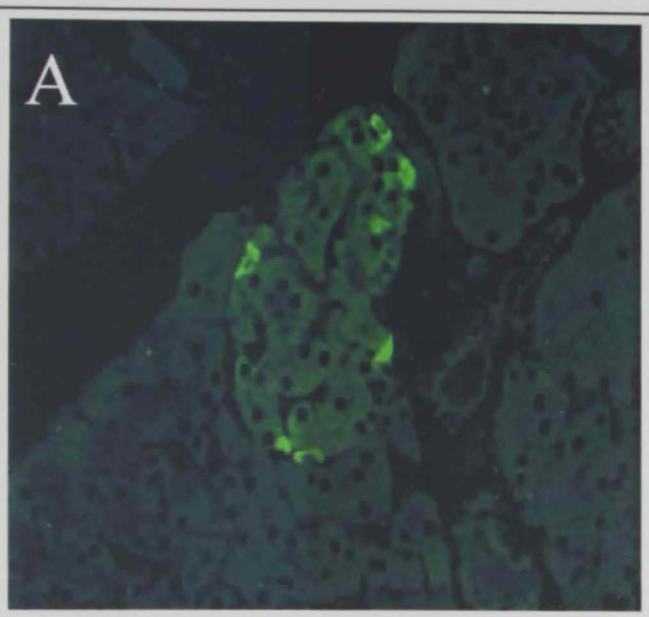
A- PP (Alone)

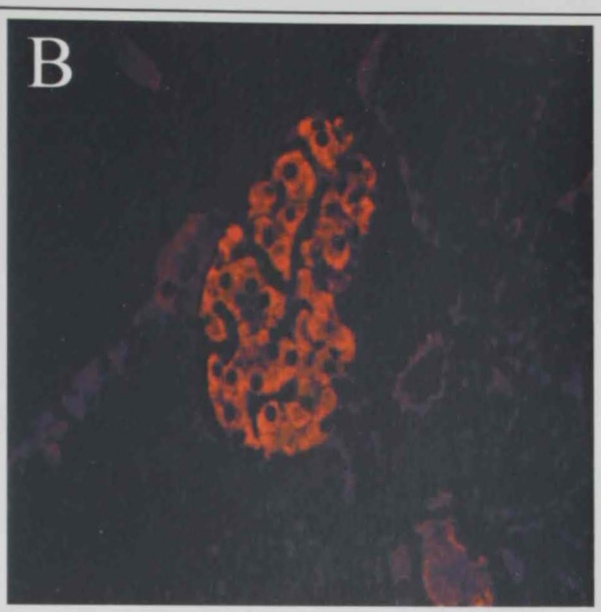
B- Visfatin (Alone)

C- Merged

*Note that the number of PP-positive cells is high. Many islet cells contain both PP and visfatin

**Magnification: X 200**



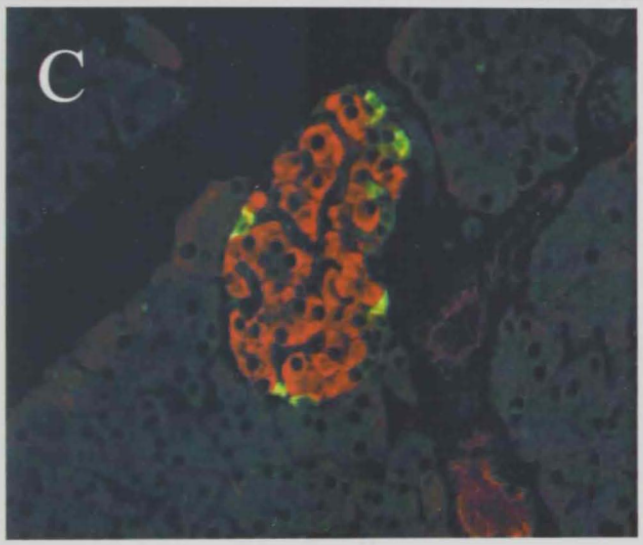




Figure (54) representative micrographs showing PP (Green) and visfatin- positive cell (Red) in pancreas islets of normal Wistar rats.

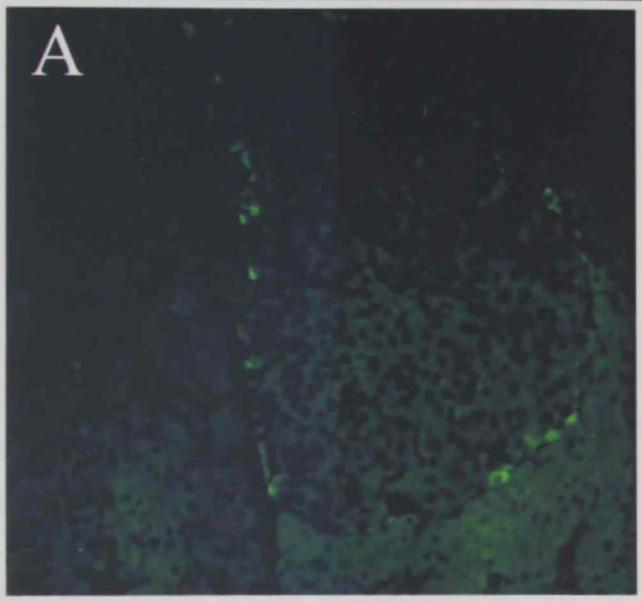
A- PP (Alone)

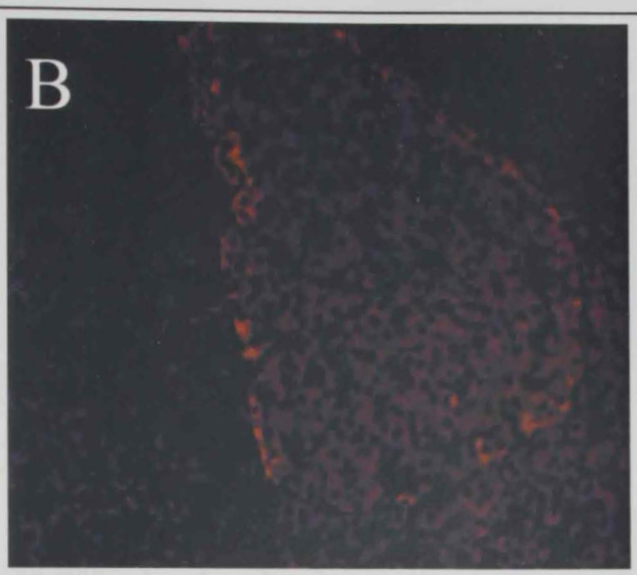
B- Visfatin (Alone)

C- Merged

*Note that cells in the periphery of the islets contain both visfatin and PP

**Magnification: X 200**



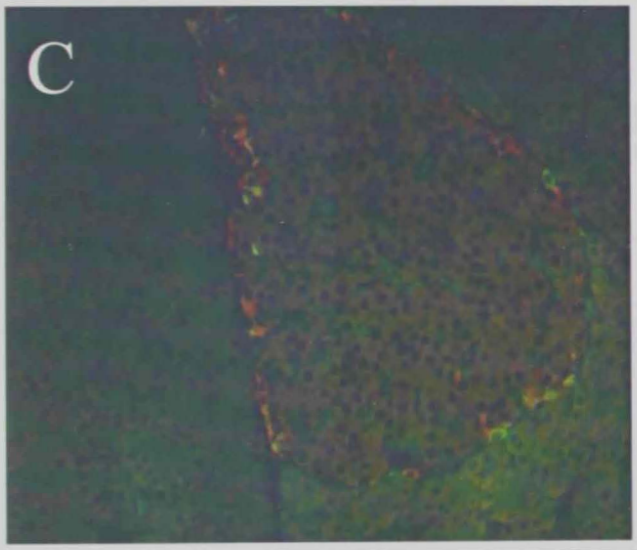




Figure (55) representative micrographs showing PP cell (Green) and visfatin- positive cell (Red) in pancreas islets of normal Wistar rats treated with visfatin

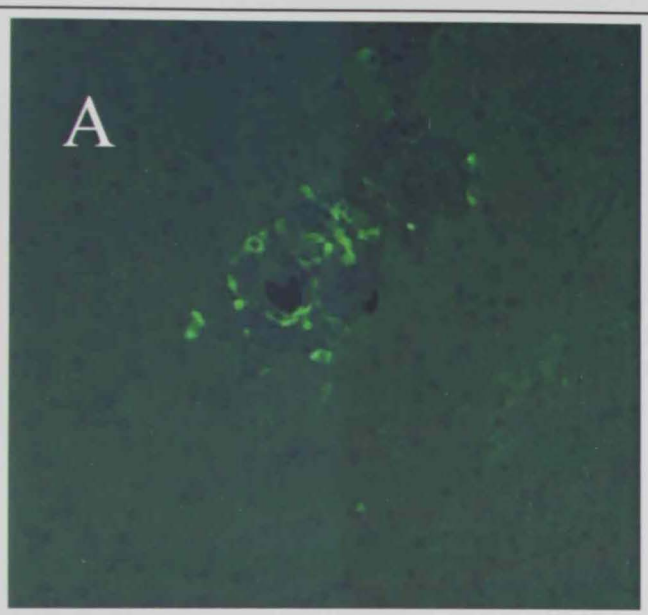
A- PP (Alone)

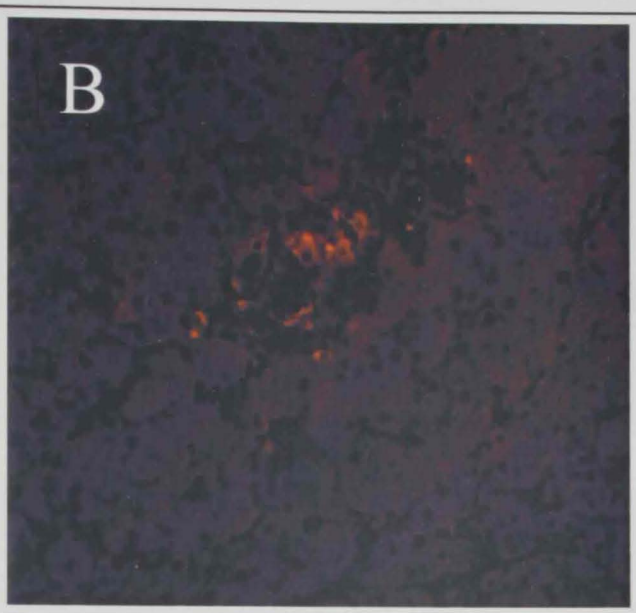
B- Visfatin (Alone)

C- Merged

*Note that cells in the periphery of the islets contain both visfatin and PP

**Magnification: X 200**



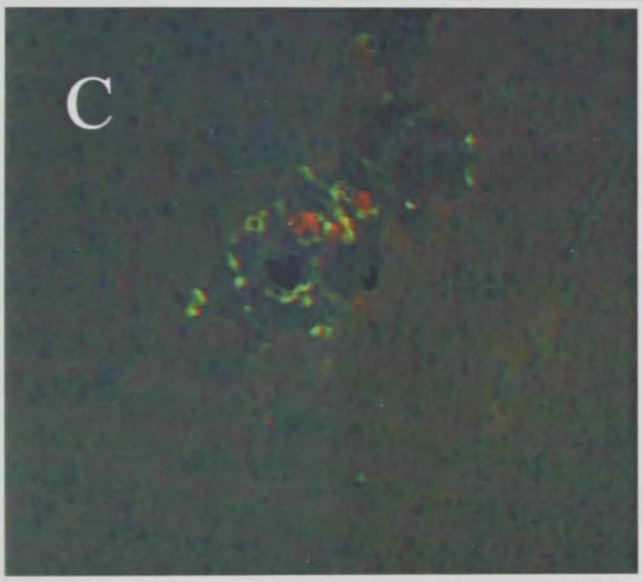




Figure (56) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and Visfatin positive cell (Red) in pancreas islets of diabetic un-treated Wistar rats.

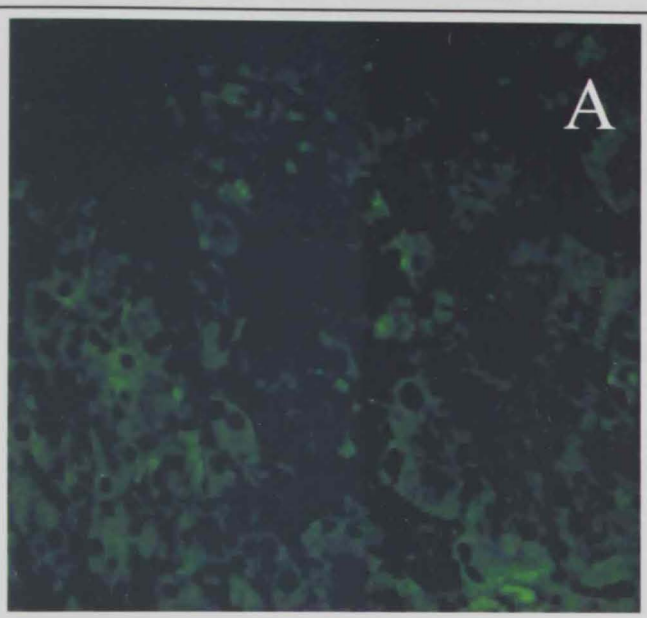
A- PP (Alone)

B- Visfatin (Alone)

C- Merged

*Note that the number of visfatin-positive cells decreased in diabetic rats.

**Magnification: X 200**



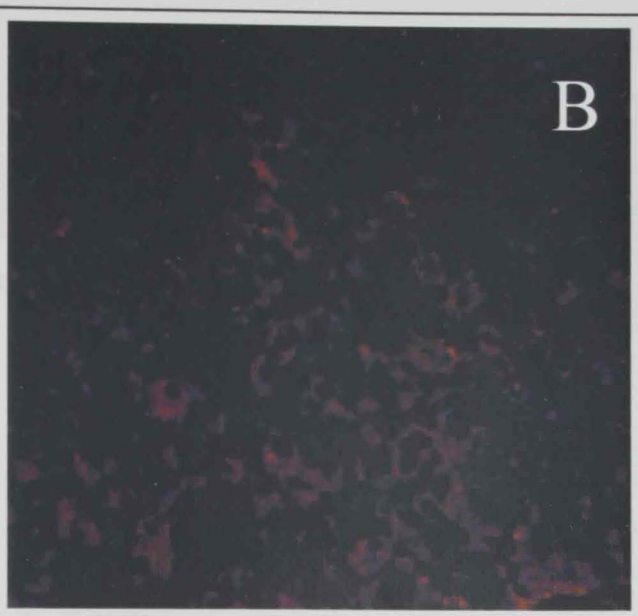





Figure (57) representative micrographs showing pancreatic polypeptide (PP) positive cell (Green) and Visfatin positive cell (Red) in pancreas islets of diabetic treated Wistar rats.

A- PP (Alone)

B- Visfatin (Alone)

C- Merged

*Note that many islet cells contain both visfatin and PP.

**Magnification: X 200**

## III.3. Immunofluorescence studies of adiponectin and visfatin in subcutaneous and visceral fat

In order to attest whether adiponectin and visfatin are localized to fat tissue, we stained for adiponectin and visfatin in subcutaneous fat. The expression of adiponectin is strongest in the subcutaenous fat of diabetic rat compared to non-diabetic normal Wistar rats (Figure 58). In a similar manner, the expression of visfatin was strongest in the subcutaneous fat of diabetic Wistar and GK rats (Figure 59).

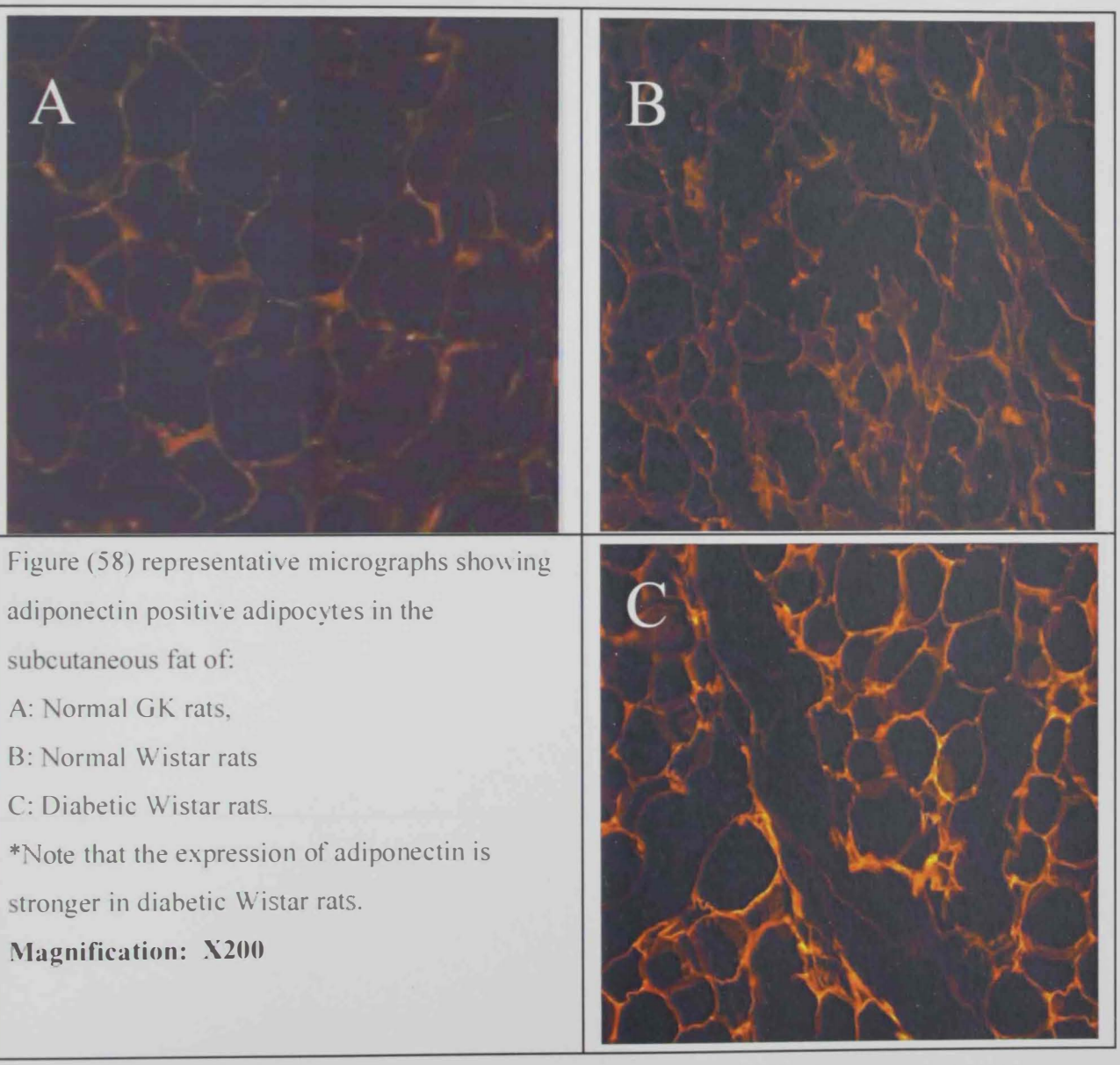
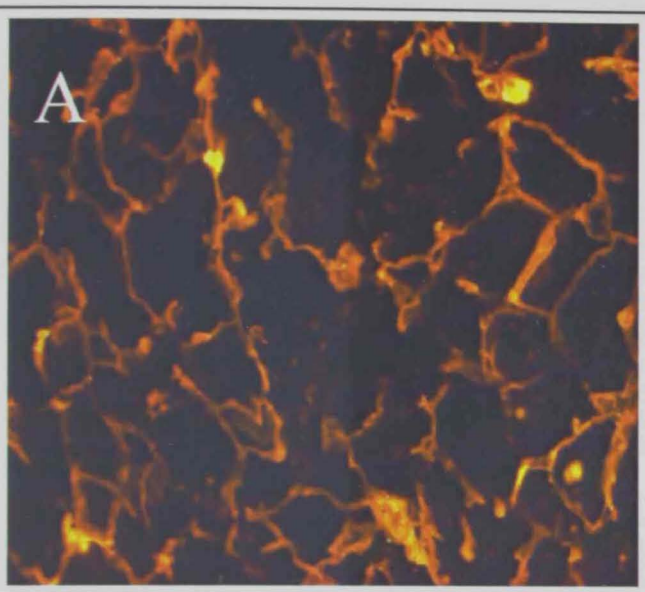



Figure (58) representative micrographs showing adiponectin positive adipocytes in the subcutaneous fat of:
A: Normal GK rats,
B: Normal Wistar rats
C: Diabetic Wistar rats.
*Note that the expression of adiponectin is stronger in diabetic Wistar rats.
**Magnification: X200**



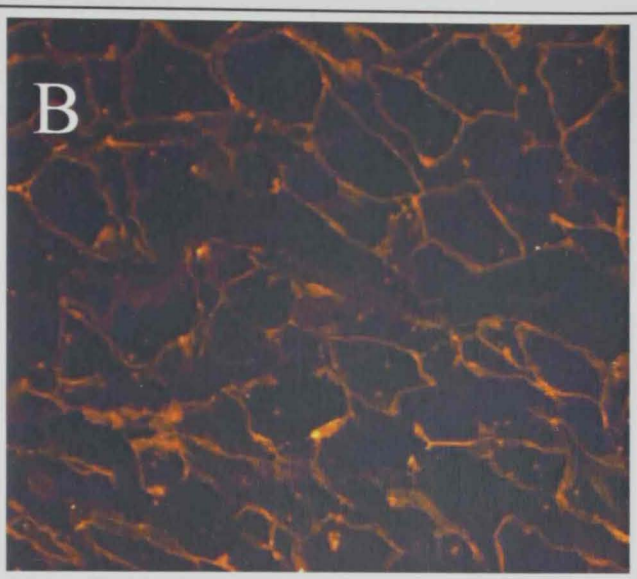




Figure (59) representative micrographs showing visfatin positive adipocytes in the subcutaneous fat of:

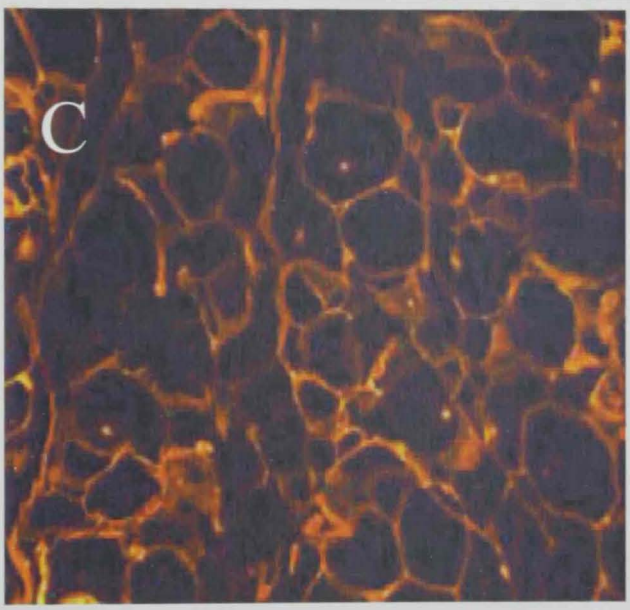
A: Normal GK rats

B: Normal Wistar rats

C: Diabetic Wistar rats.

*Note that the expression of visfatin is stronger in diabetic (A, C) rats.

**Magnification: X 200**



Adiponectin-immunopositive adipocytes in the epididymal fat of untreated GK, normal Wistar and diabetic Wistar in figure 60. The pattern and intensity of adiponectin expression appears to be similar in all groups of animal tested. In contrast, the expression of visfatin appears to be strongest in the epididymal fat of diabetic Wistar rats (Figure 61).

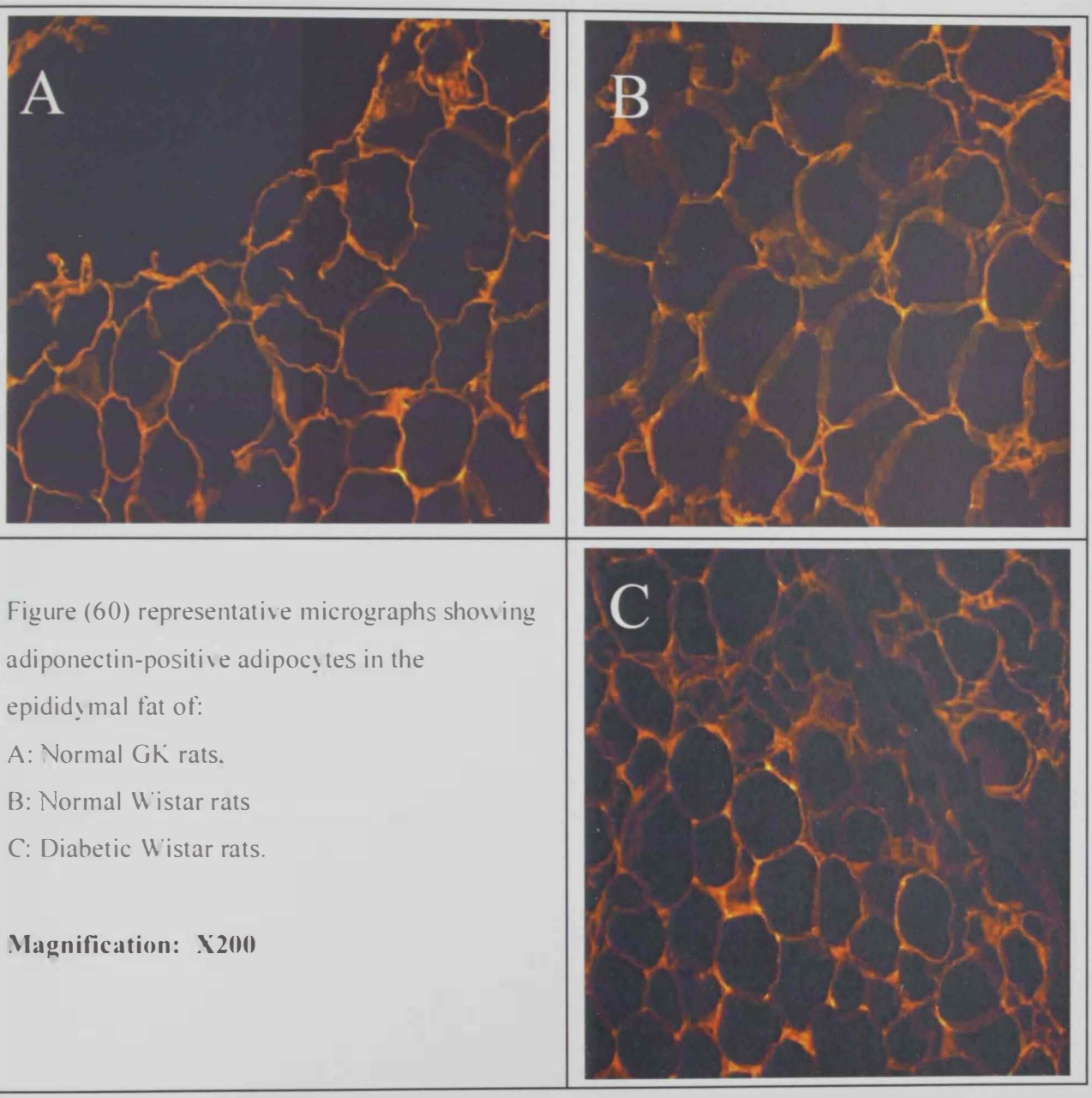
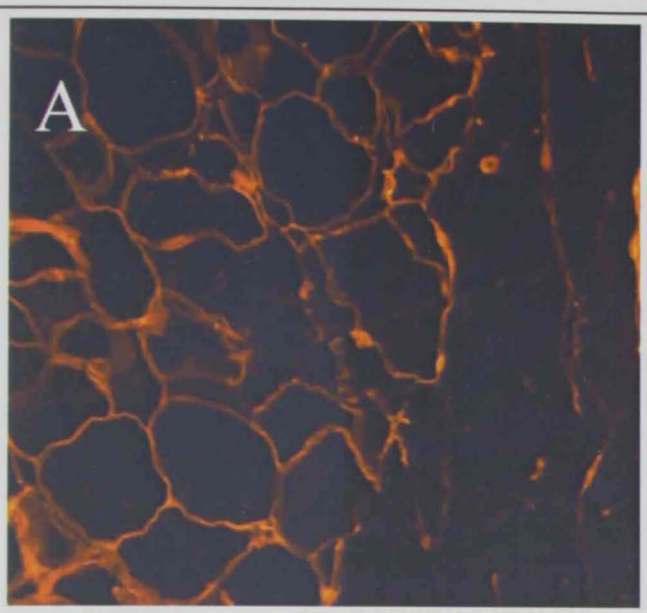



Figure (60) representative micrographs showing adiponectin-positive adipocytes in the epididymal fat of:
A: Normal GK rats.
B: Normal Wistar rats
C: Diabetic Wistar rats.

**Magnification: X200**



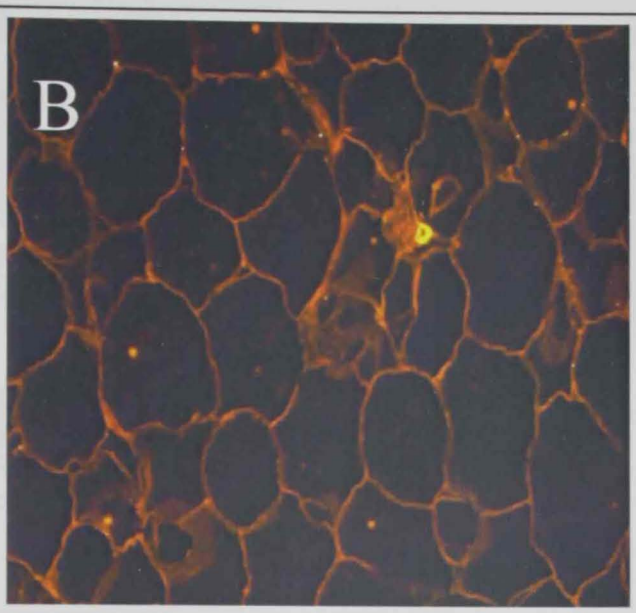





Figure (61) representative micrographs showing visfatin-positive adipocytes in the epididymal fat of

A: Normal GK rats

B: Normal Wistar rats

C: Diabetic Wistar rats.

*Note that visfatin appears to be more expressed in visceral fat of diabetic Wistar rats.

**Magnification: X200**

## IV. Effect of adiponectin and visfatin on islet morphology

Figures (62.B, 63.B, 64.B) show that the islets of GK, normal and diabetic Wistar rats treated with adiponectin are larger than those of control Figures (62.A, 63.A, 64.A)

In a similar trend, visfatin increased the size of pancreatic islets in GK, normal and diabetic Wistar rats after treatment for 14 weeks Figures (65.B, 66.B, 67.B)

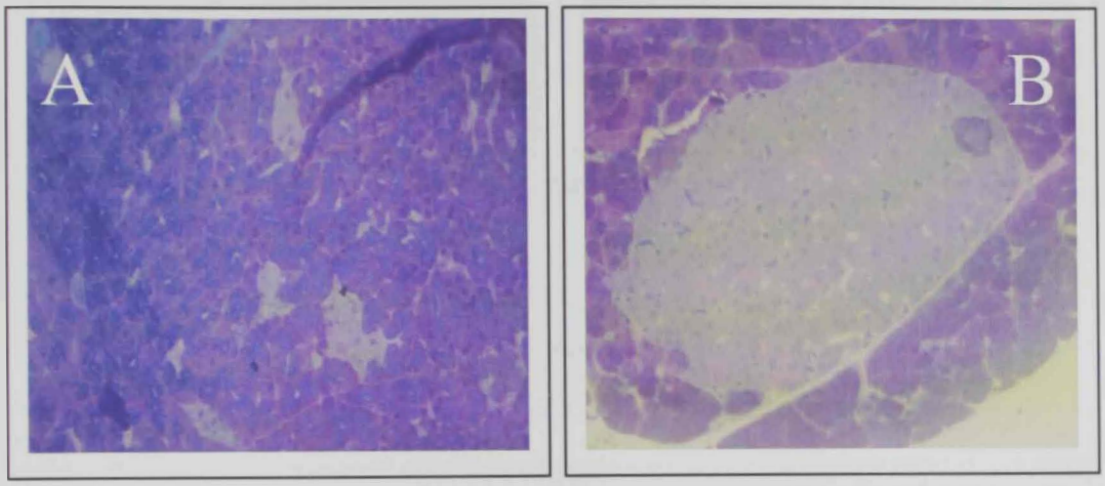



Figure (62) representative micrographs showing islets in:

A- Normal GK rats

B- GK rats treated with adiponectin

*Note that a large increase in the size of islets in treated rats versus control.

**Magnification: X 400**

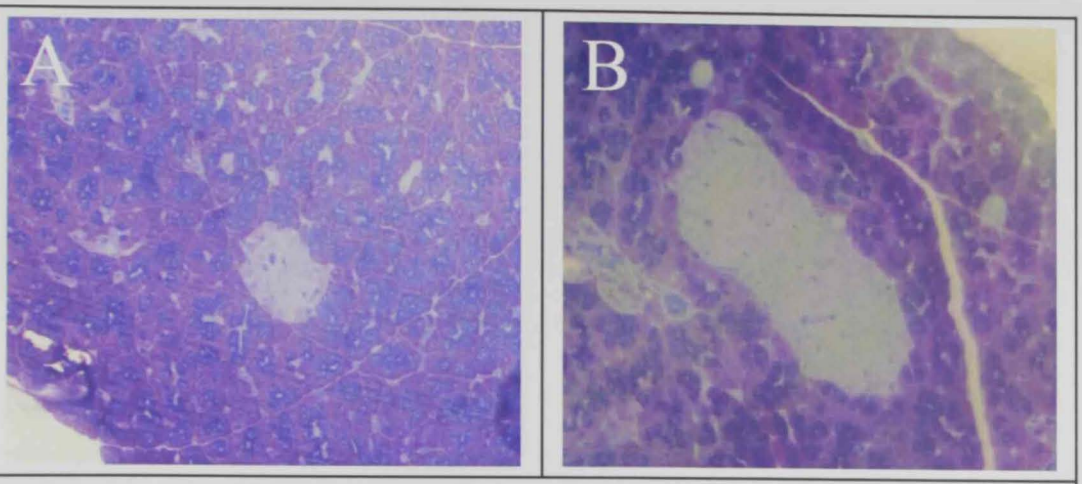



Figure (63) representative micrographs showing islets in:

A- Normal Wistar rats

B- Normal Wistar rats treated with adiponectin

*Note an increase in the size of islets in treated rats versus control.

**Magnification: X 400**

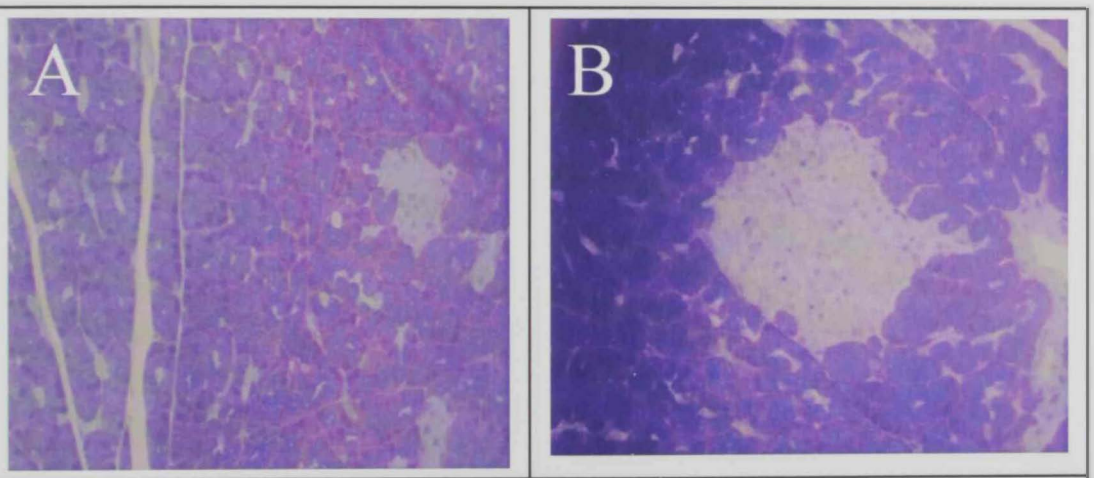



Figure (64) representative micrographs showing islets of:

A- Diabetic Wistar rats

B- Diabetic Wistar treated with Adiponectin

*Note that the large islet in the diabetic treated rats shows a conspicuous recovery.

**Magnification: X 400**

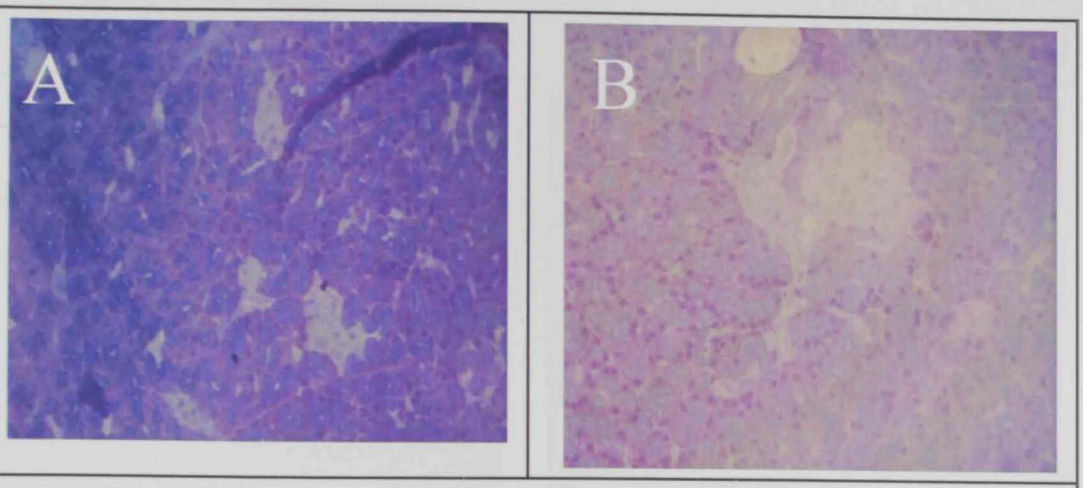



Figure (65) representative micrographs showing islets in:

A- Normal GK rats

B- GK treated with visfatin

*Note a small increase in the size of islets of treated rats.

**Magnification: X 400**

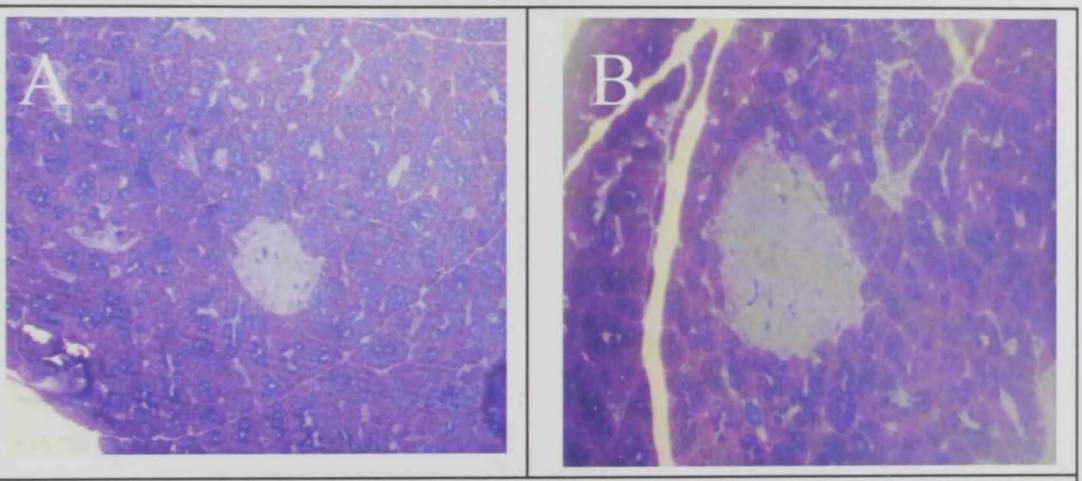



Figure (66) representative micrographs showing islets in:

A- Normal Wistar rats

B- Normal Wistar rats treated with visfatin

*Note that the size of islets in treated rats is larger compared to control.

**Magnification: X 400**

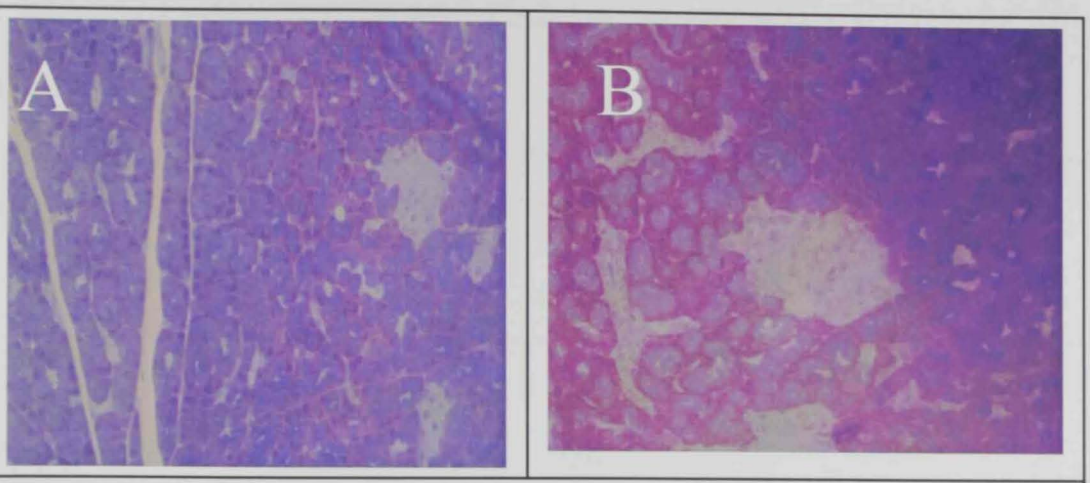



Figure (67) representative micrographs showing islets of:

A- Diabetic Wistar rats

B- Diabetic Wistar treated with visfatin

There is a small increase in the size of islets in treated group compared to control.

**Magnification: X 400**

## V. Effect of adiponectin and visfatin on the ultra structure of pancreatic beta cells

Islet morphology was significantly improved in GK, normal and diabetic Wistar rats treated with either adiponectin or visfatin. Figures (68-73)

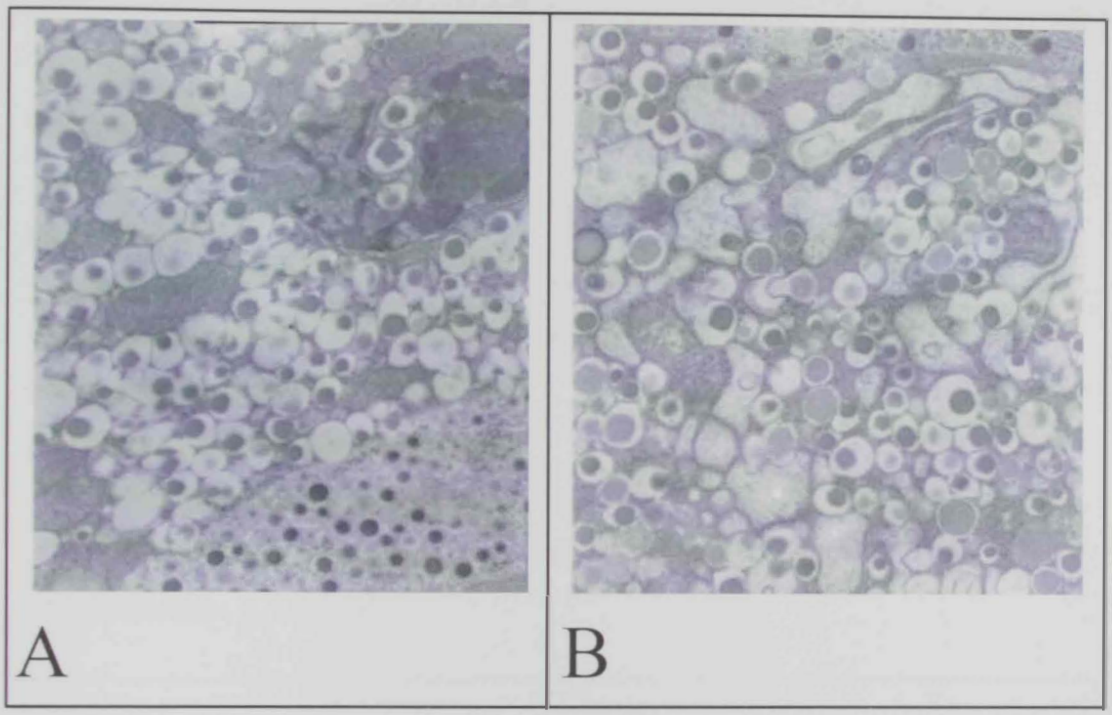


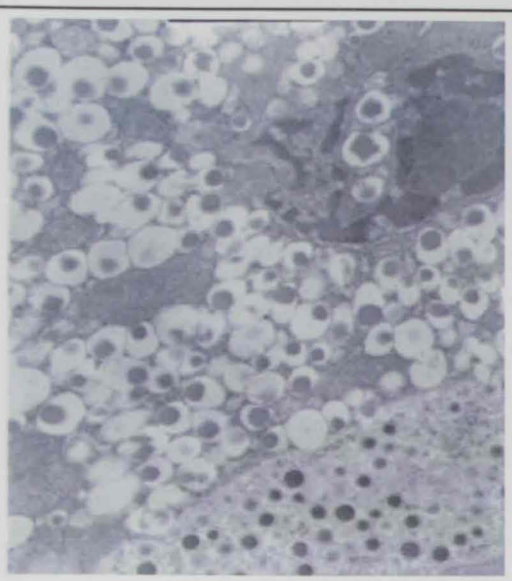

Figure (68) representative micrographs showing ultra structure of pancreatic beta cells.

A. Pancreatic beta cells in GK control

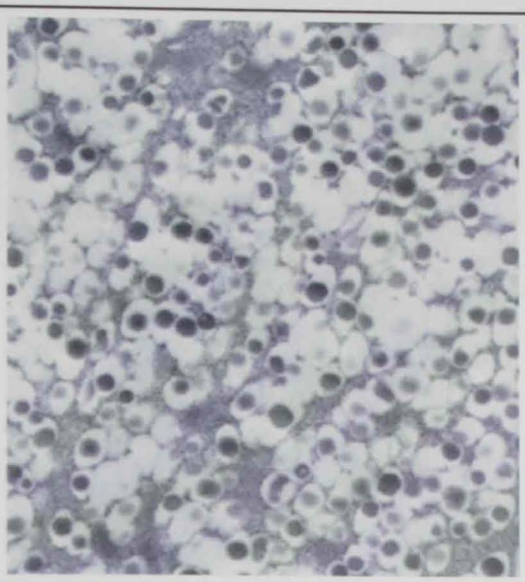
B. Pancreatic beta cells in GK treated with adiponectin.

*Note the large increase in pancreatic beta cell granule.

**Magnification: X 14, 5000**



A



B

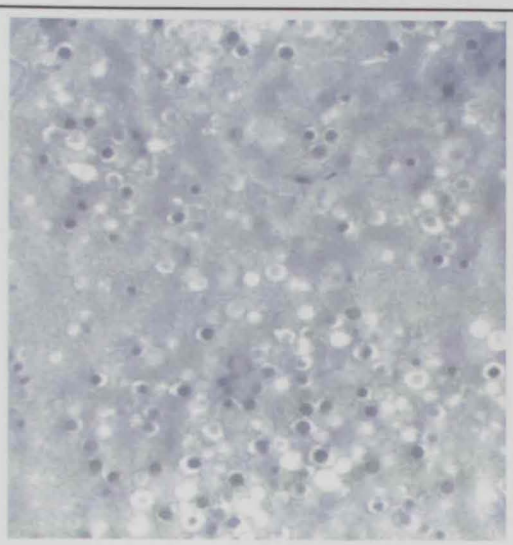
Figure (69) representative micrographs showing ultra structure of pancreatic beta cells.

A. Pancreatic beta cells in GK control

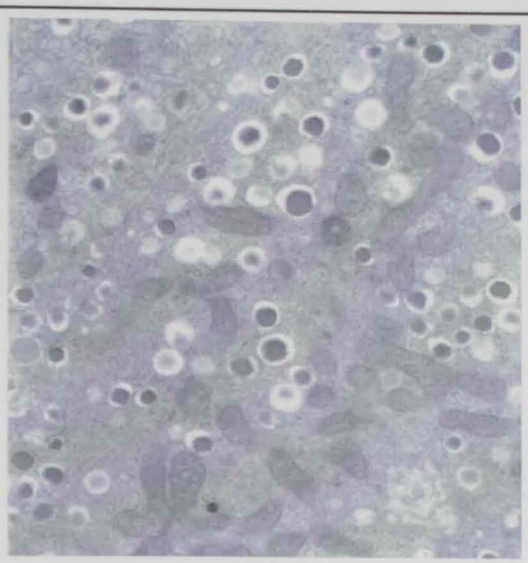
B. Pancreatic beta cells in GK treated with visfatin.

* Note the large increase in pancreatic beta cell granule.

**Magnification: X 14, 5000**



A



B

Figure (70) representative micrographs showing ultra structure of pancreatic beta cells.

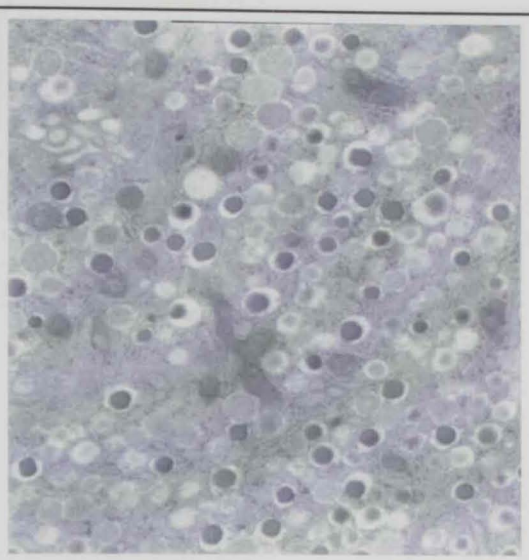
A.Pancreatic beta cells in normal Wistar

B. Pancreatic beta cells in normal Wistar treated with adiponectin.

*Note the large increase in pancreatic beta cell granule

**Magnification: X14, 5000**

A



B

Figure (71) representative micrographs showing ultra structure of pancreatic beta cells.

A. Pancreatic beta cells in normal Wistar

B. Pancreatic beta cells in normal Wistar treated with visfatin.

*Note a large increase in pancreatic beta cell granule

**Magnification: X14, 5000**

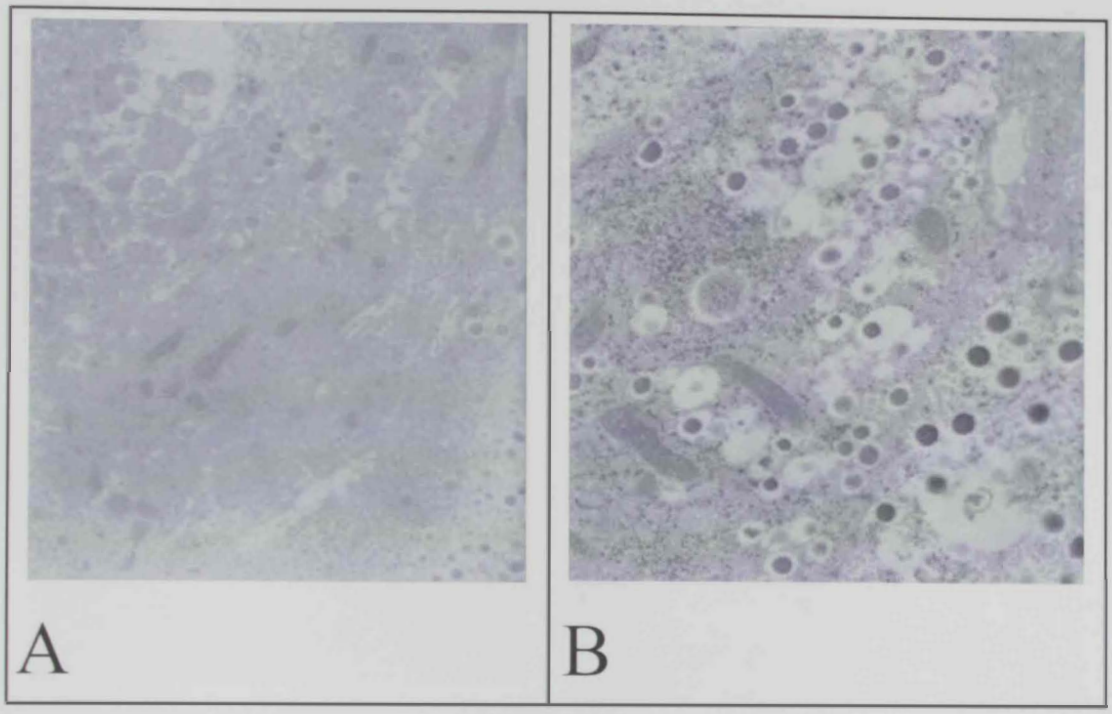


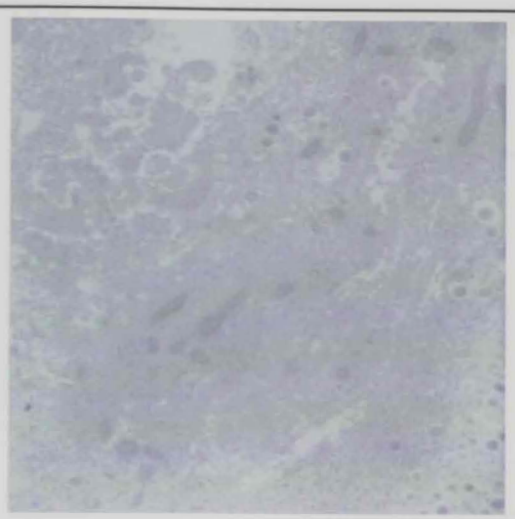

Figure (72) representative micrographs showing ultra structure of pancreatic beta cells.

A.Pancreatic beta cells in untreated diabetic

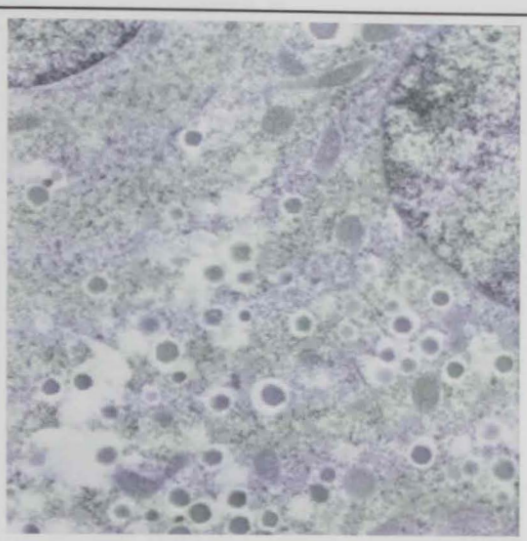
B. Pancreatic beta cells in Adiponectin -treated Wistar rats.

*Note that a large increase in pancreatic beta cell granule of treated rats

**Magnification: X14, 5000**



A



B

Figure (73) representative micrographs showing ultra structure of pancreatic beta cells.

A. Pancreatic beta cells in untreated diabetic

B. Pancreatic beta cells in Visfatin-treated Wistar rats.

*Note that a large increase in pancreatic beta cell granule of treated rats.

**Magnification: X14, 5000**

## VI. Immunoelectron microscopy of adiponectin and visfatin in pancreatic islet cells

Adiponectin and visfatin were observed in secretory granules of pancreatic islet cells in GK well as Wistar rats. Many secretory granules contain both adiponectin and insulin or visfatin and insulin. Some adiponectin and visfatin-postive gold particles were also observed on the rough endoplasmic reticulum. (Figures 74-75)

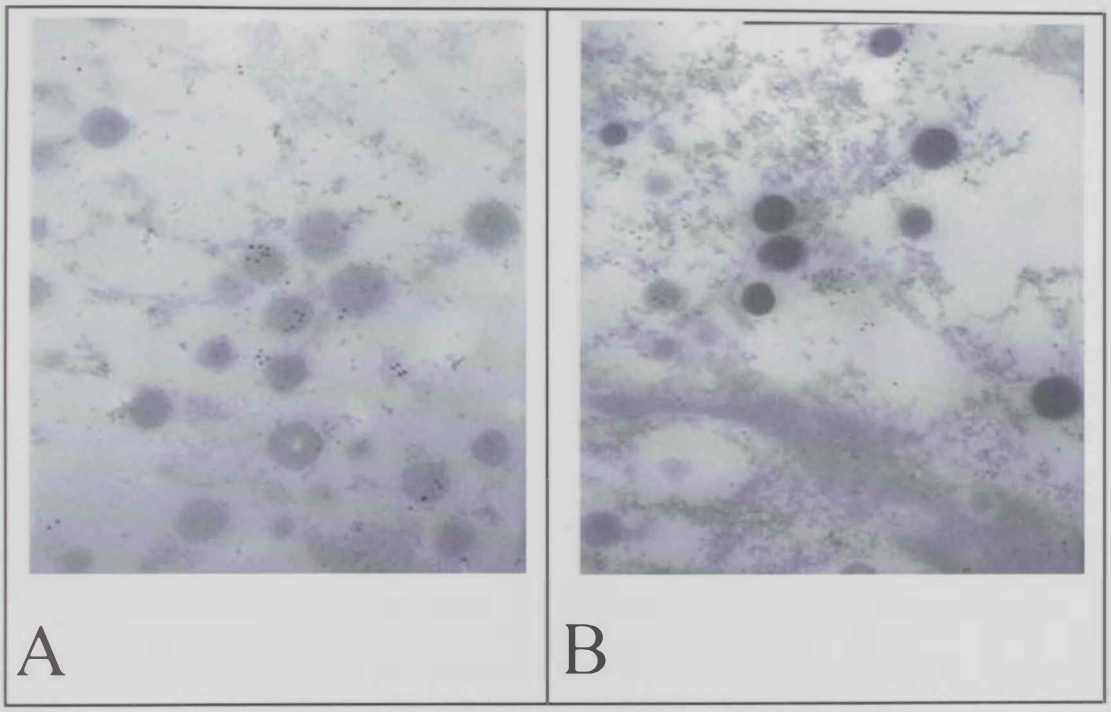
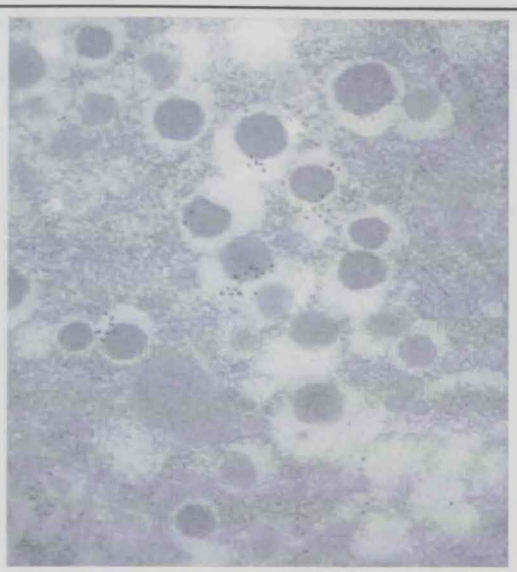
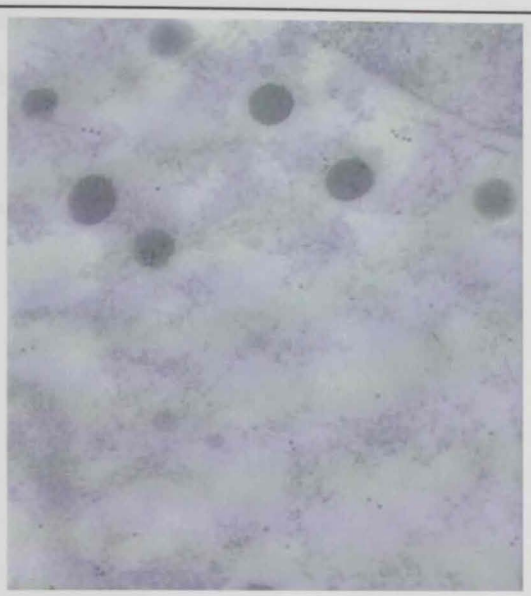



Figure (74) representative subcellular localization of adiponectin and insulin (A) and visfatin and insulin in pancreatic islet cells of GK rats (B)
*Note that insulin (10 nm gold particles) and visfatin (20 nm gold particles) co-localize with (A) adiponectin (20 nm gold particles)and (B) visfatin
**Magnification: X 35, 000.**



A



B

Figure (75) representative subcellular localization of adiponectin and insulin (A) and visfatin and insulin in pancreatic islet cells of normal Wistar rats. (B)
*Note that insulin (10 nm gold particles) and visfatin (20 nm gold particles) co-localize with adiponectin (20 nm gold particles) (A) and (B) visfatin.
**Magnification: X 35, 000.**

## VII. Plasma insulin and glucagon level of GK, normal and Diabetic Wistar rats after adiponectin and visfatin treatment

Normal Wistar rats treated with visfatin showed a significant($p < 0.05$) decrease in plasma insulin level compared to adiponectin treated and untreated control. In contrast, the insulin level of diabetic Wistar rats treated with either adiponectin or visfatin increased significantly ($p < 0.05$). In addition, adiponectin increased level while visfatin decreased plasma insulin in GK rats(Figures 76-78).

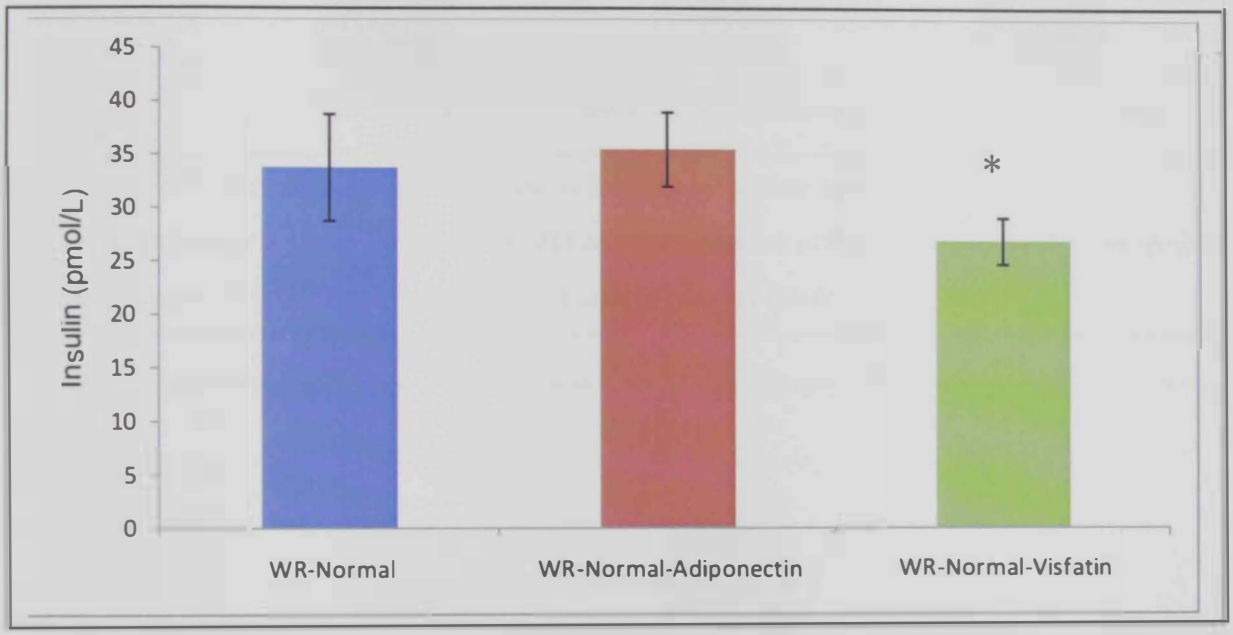



Figure (76) shows plasma insulin levels in normal Wistar rats treated with either adiponectin or visfatin. Normal Wistar rats treated with visfatin show significant ($p < 0.05$) decrease in plasma insulin level.

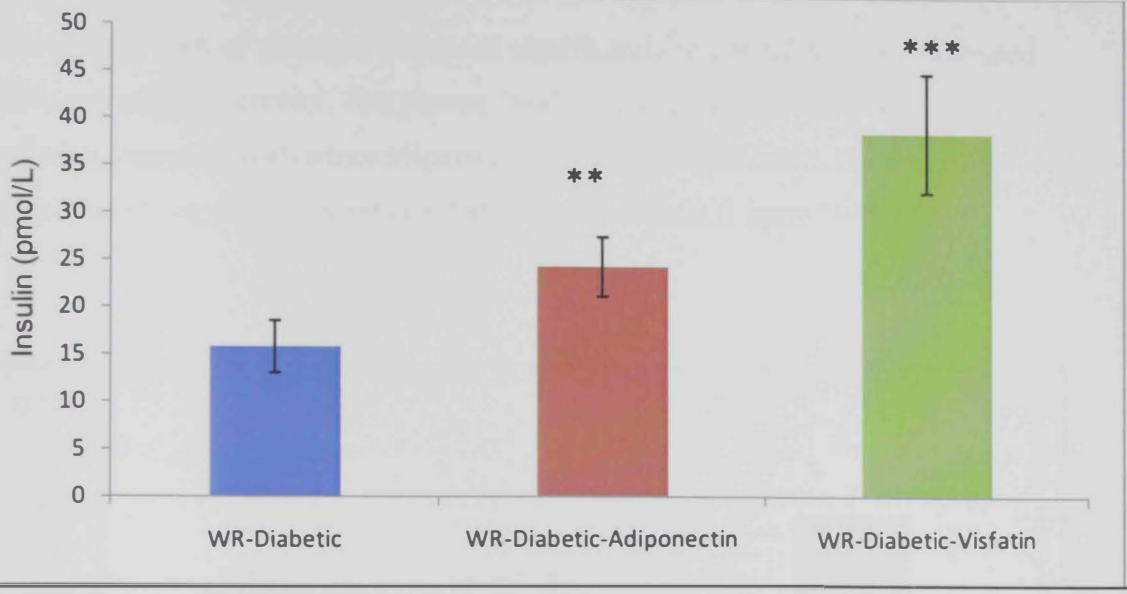



Figure (77) shows plasma insulin levels in diabetic Wistar rats treated with either adiponectin or visfatin. Diabetic Wistar rats treated with either adiponectin or visfatin show significant ($p < 0.005$); $p < 0.0005$) increase in plasma insulin level.

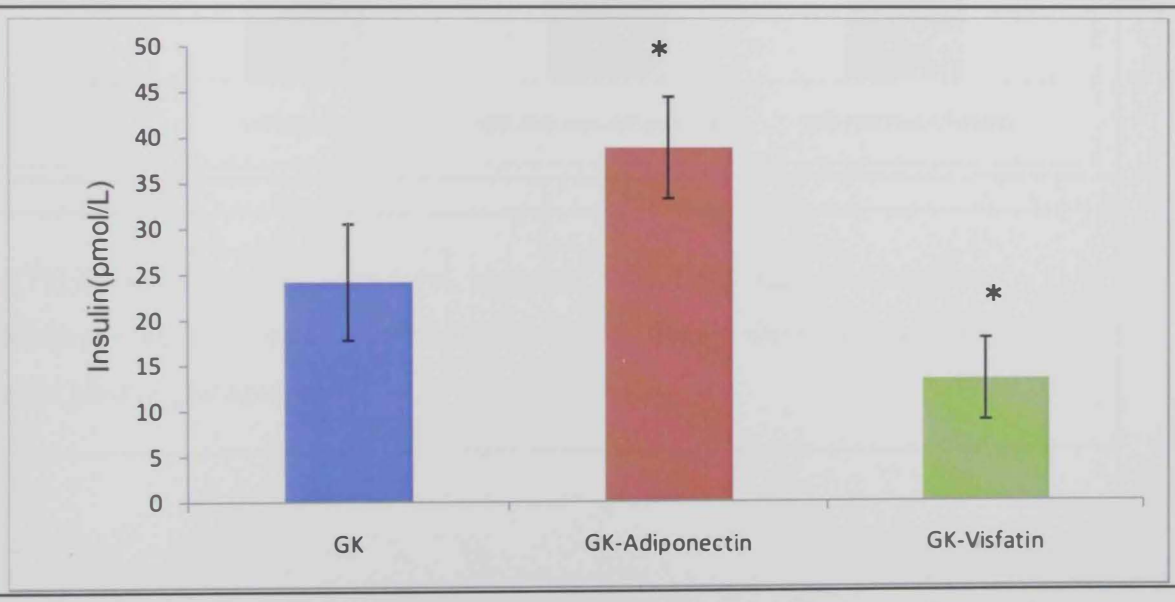



Figure (78) shows plasma insulin levels in GK rats treated with either adiponectin or visfatin. GK rats treated with adiponectin showed a significant increase in plasma insulin level while those treated with visfatin have significant ($p < 0.05$) decrease in plasma insulin level.

The plasma level of glucagon increased significantly in normal Wistar rats treated with visfatin compared to control. The plasma level of glucagon in diabetic Wistar rats was not altered after treatment with either adiponectin or visfatin. In contrast, the plasma level of glucagon decreased sharply in GK rats treated with adiponectin (Figure 79-81)

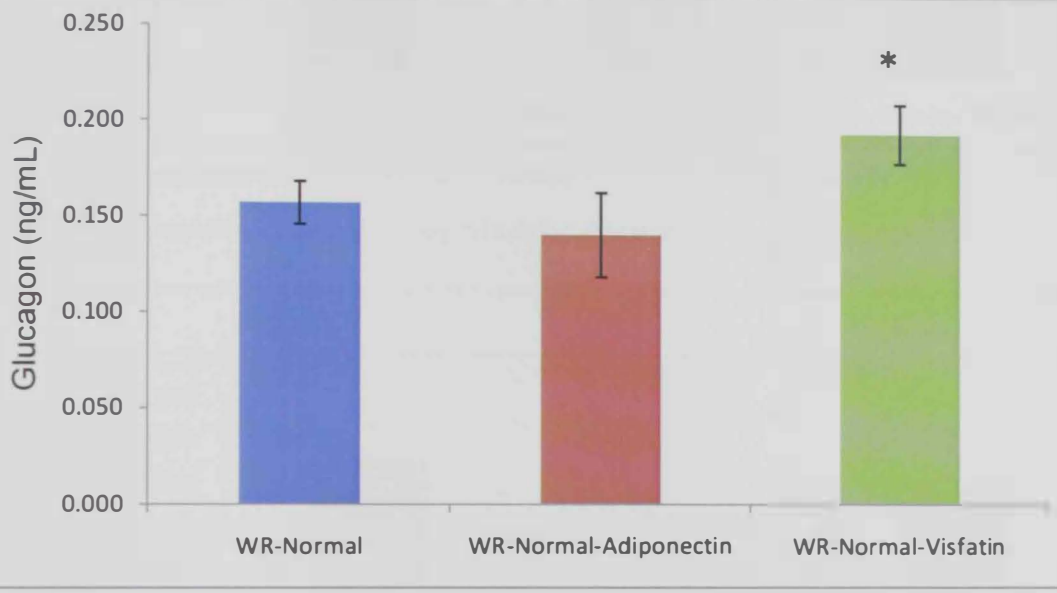



Figure (79) shows plasma glucagon level in normal Wistar rats treated with either adiponectin or visfatin. Normal Wistar rats treated with visfatin show significant ($p < 0.05$) increase in plasma glucagon level.

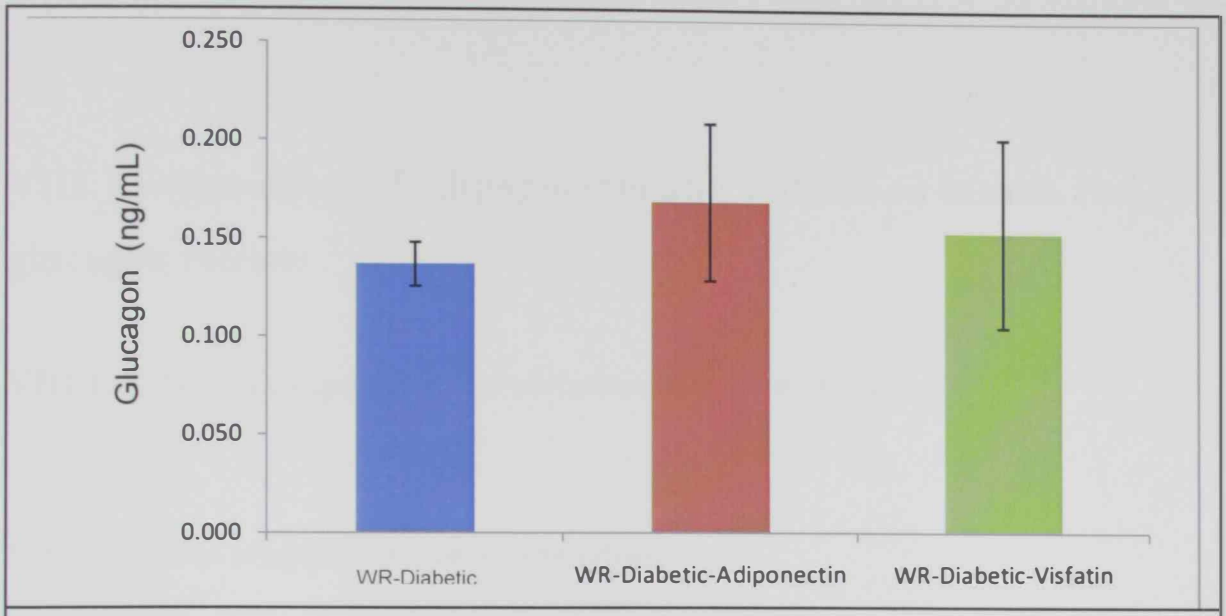



Figure (80) shows plasma glucagon level in diabetic Wistar rats treated with either adiponectin or visfatin. There is no significant difference between the 3 groups.

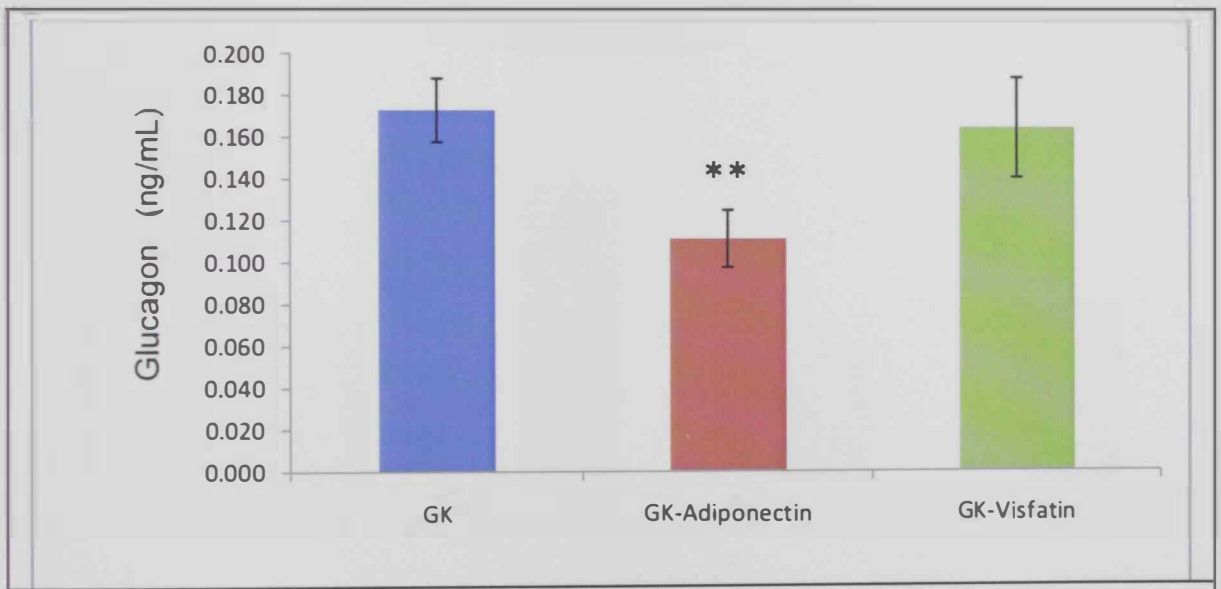



Figure (81) shows plasma glucagon level in GK rats treated with either adiponectin or visfatin.

Note that GK Wistar rats treated with adiponectin show significant ($p < 0.005$) decrease in plasma glucagon level.

# VIII. In vitro effect of adipopnectin and visfatin on insulin and glucagon release

## VIII.1. Effect of adiponectin or visfatin on insulin release

### VIII.1. a. Effect of adiponectin on insulin release

Adiponectin inhibited insulin from the pancreas of Wistar rats at $10^{-12}$ M. No significant effect of adiponectin was observed in GK rats (Figure 82-85)

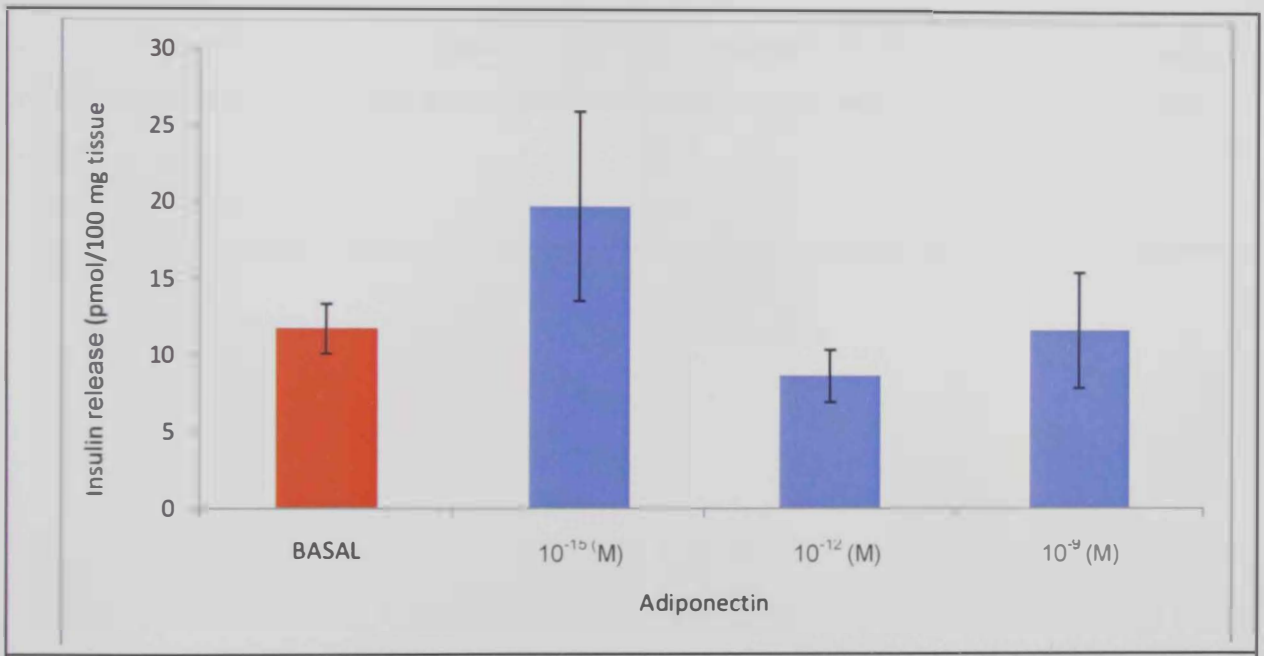



Figure (82) shows the effect of different concentrations of Adiponectin on insulin release of GK rat's pancreatic tissue.

*Note that adiponectin caused a slight but not significant increase in insulin release at $10^{-15}$ M

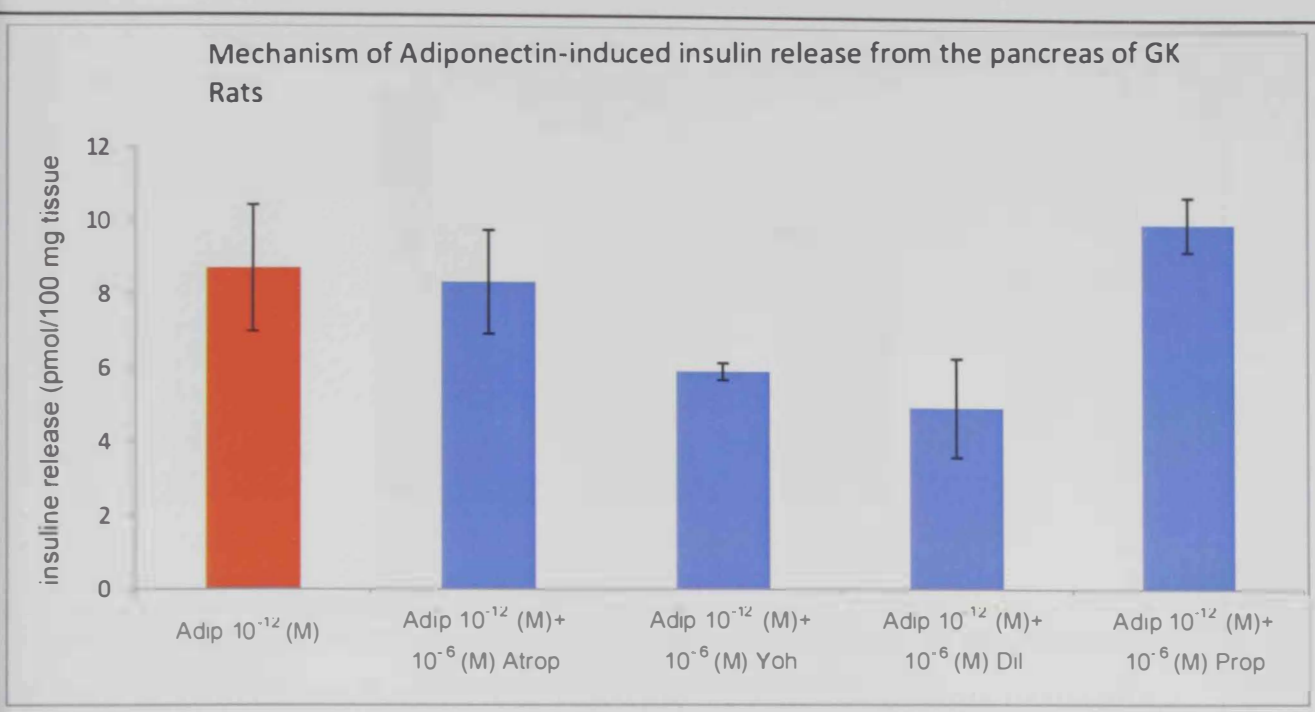



Figure (83) shows insulin release from the pancreas of normal GK rats after incubation with different receptor antagonists.

Note that neither, atropine (atrop), yohimbine (Yoh), nor propranolol (prop) affects adiponectin-induced inhibition of insulin release. Diltiazem (Dil) caused a slight but not significant decrease in insulin release.

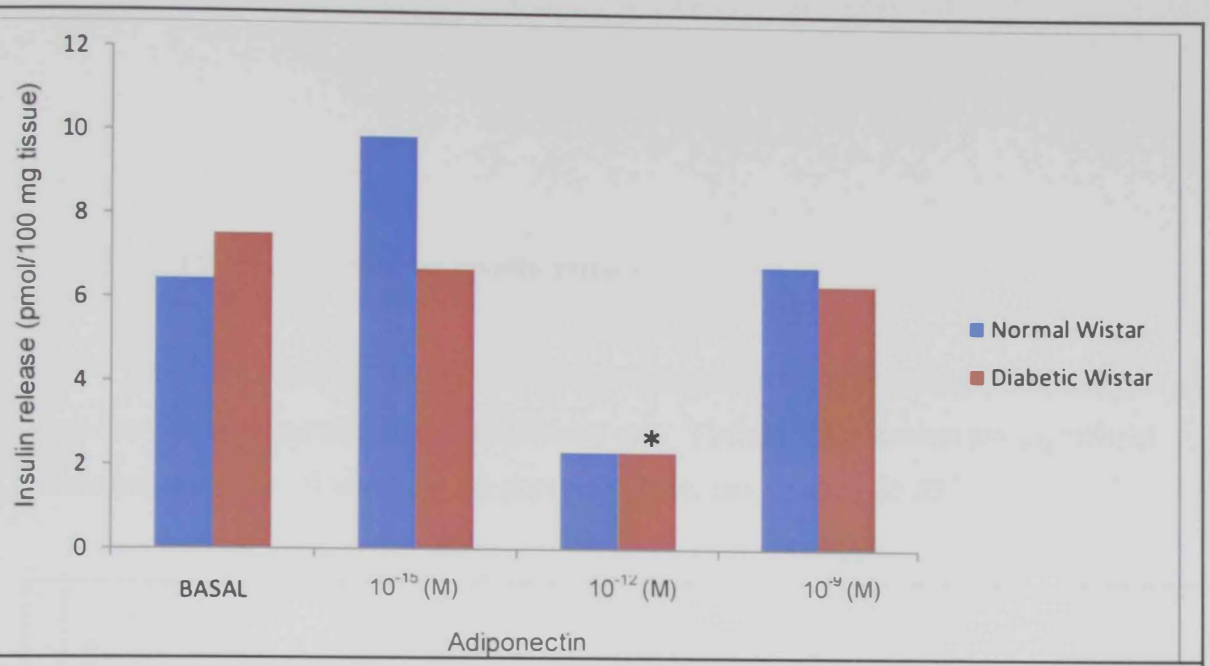



Figure (84) shows the effect of different concentrations of adiponectin on insulin release from the pancreas of normal and diabetic Wistar rats. *Note that adiponectin significantly inhibited insulin release from the pancreas of both normal and diabetic rats at $10^{-12}$ M.

* ($P < 0.05$ )

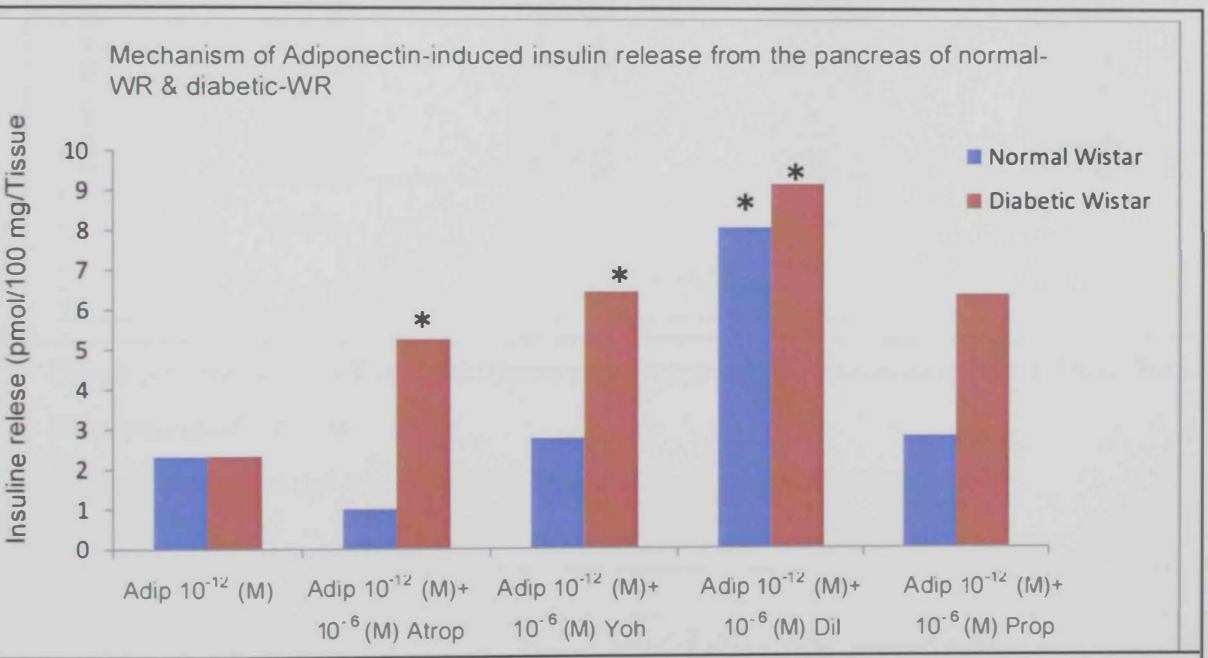



Figure (85) shows insulin release from the pancreas of normal & diabetic-Wistar rats after incubation of tissues with different receptor antagonists. *Note that incubation with either atropine (Atrop), yohimbine (Yoh), or diltiazem (Dil) caused large increases in insulin realease from the pancreas of diabetic rats.

### VIII.1.b. Effect of visfatin on insulin release

Visfatin inhibited insulin release from pancreatic tissue fragments of Wistar rats at all concentrations especially in diabetic Wistar rats. Visfatin did not cause any significant changes in insulin release from the pancreas of GK rats. (Figure 86-89)

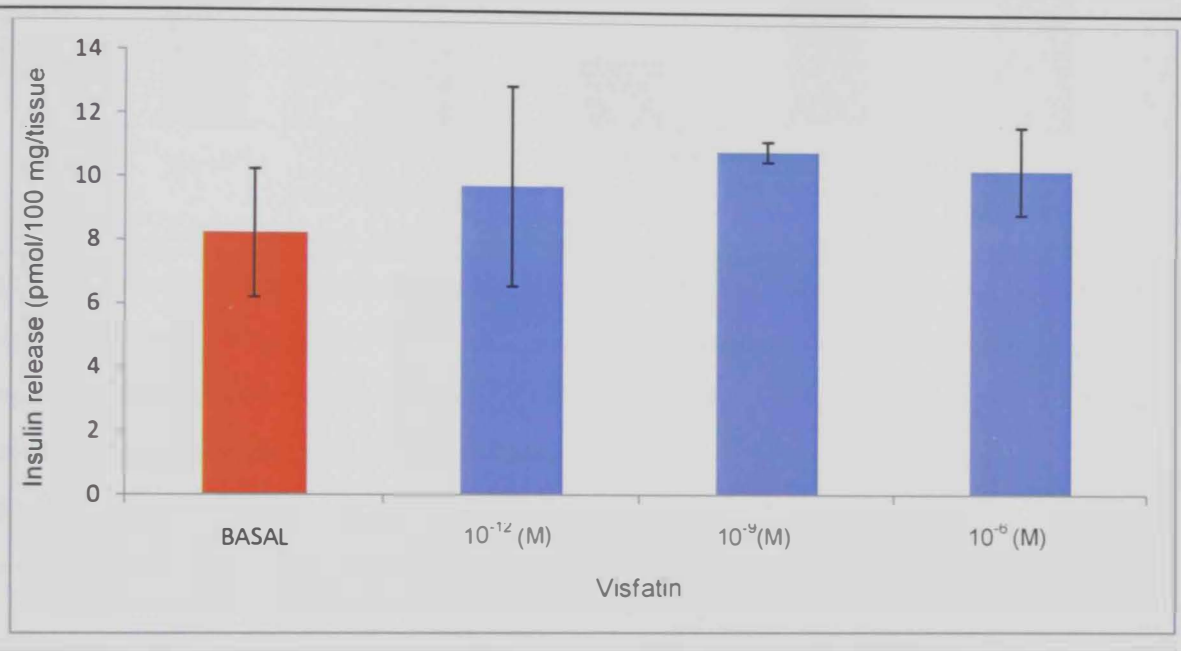



Figure (86) shows the effect of different concentrations of visfatin on insulin release from the pancreas of GK rats.

*Note that visfatin did not affect insulin release at the concentrations used.

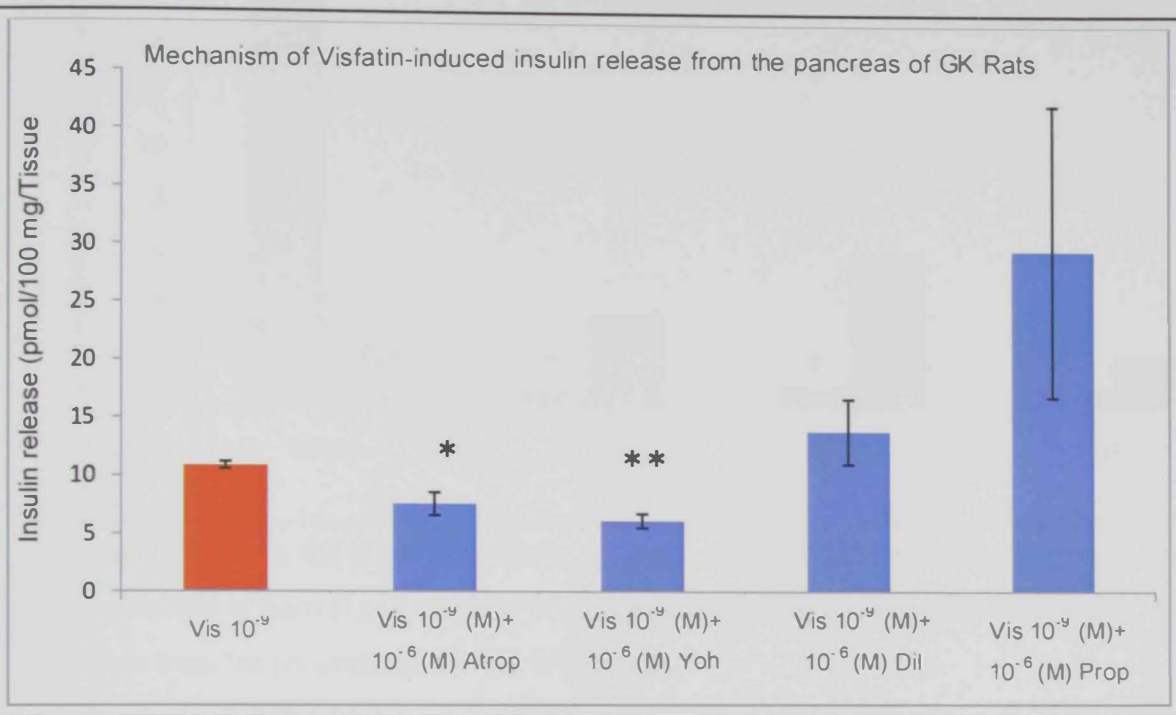



Figure (87) shows insulin release from the pancreas of normal GK rats after incubation with different receptor antagonists. *Note that neither, diltiazem (Dil) nor propranolol (prop) affects visfatin-induced insulin release. However, atropine (atrop) and yohimbine (Yoh), caused significant decreases in insulin release.

* ($p < 0.05$),**($p<0.005$)

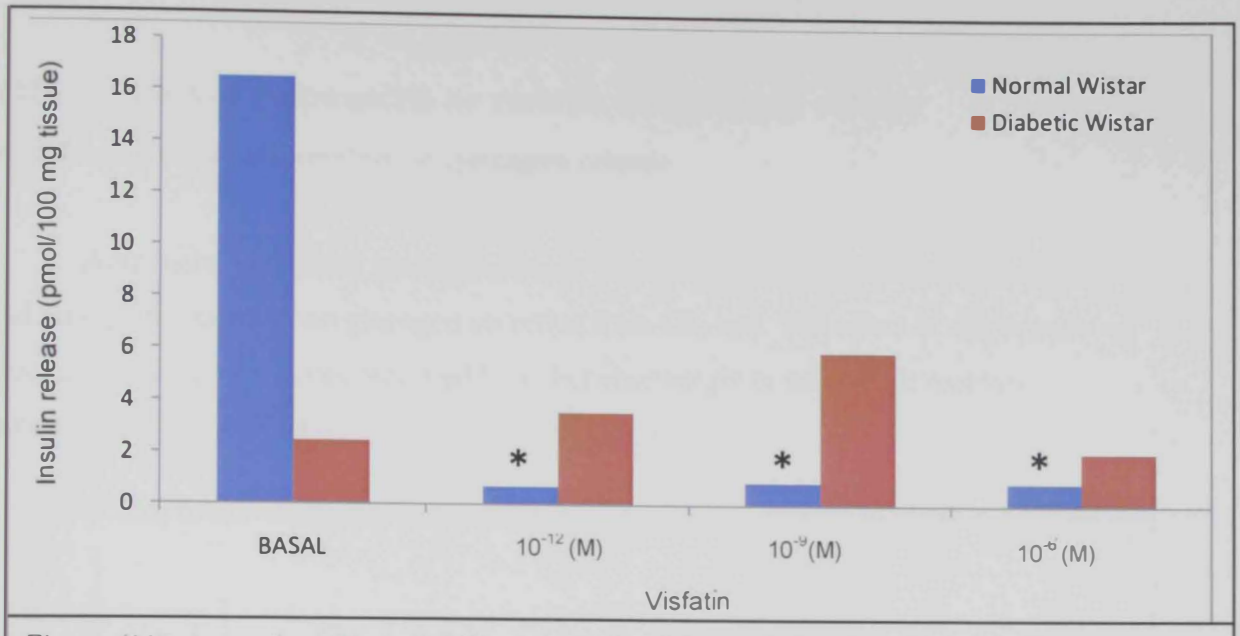



Figure (88) shows the effect of different concentrations of visfatin on insulin release from the pancreas of normal and diabetic Wistar rats.* Note that visfatin inhibits ($p \leq 0.05$) insulin release from the pancreas of normal Wistar rats.

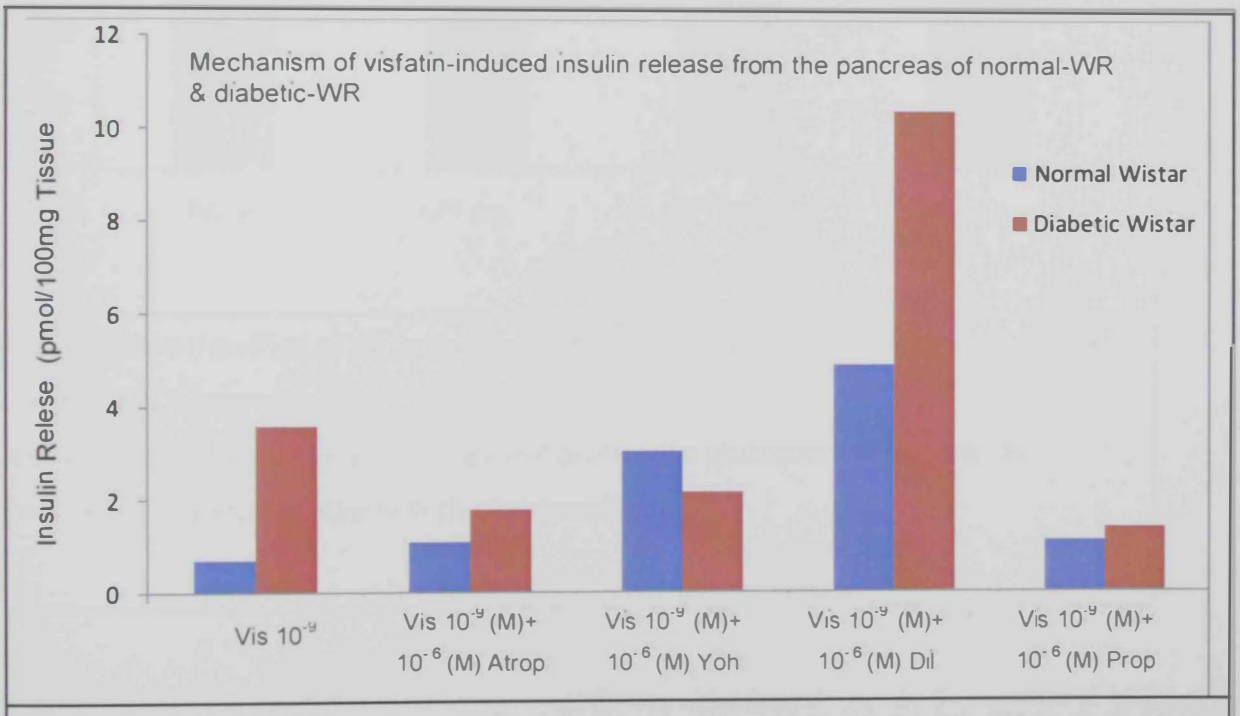



Figure (89) shows insulin release from the pancreas of normal and diabetic Wistar rats after incubation of tissues with visfatin and different receptor antagonists.

*Note that incubation with diltiazem (Dil) caused large increases in insulin release from the pancreas of both normal and diabetic rats.

## VIII.2. Effect of adiponectin or visfatin on glucagon release

### VIII.2.a. Effect of adiponectin on glucagon release

Adiponectin inhibited glucagon release from the pancreas of normal Wistar rats but had no significant effect on glucagon secretion from GK rats. The effect of adiponectin on glucagone secretion was not modified by either cholinergic or adrenergic receptor antagonists. (Figure 90-93)

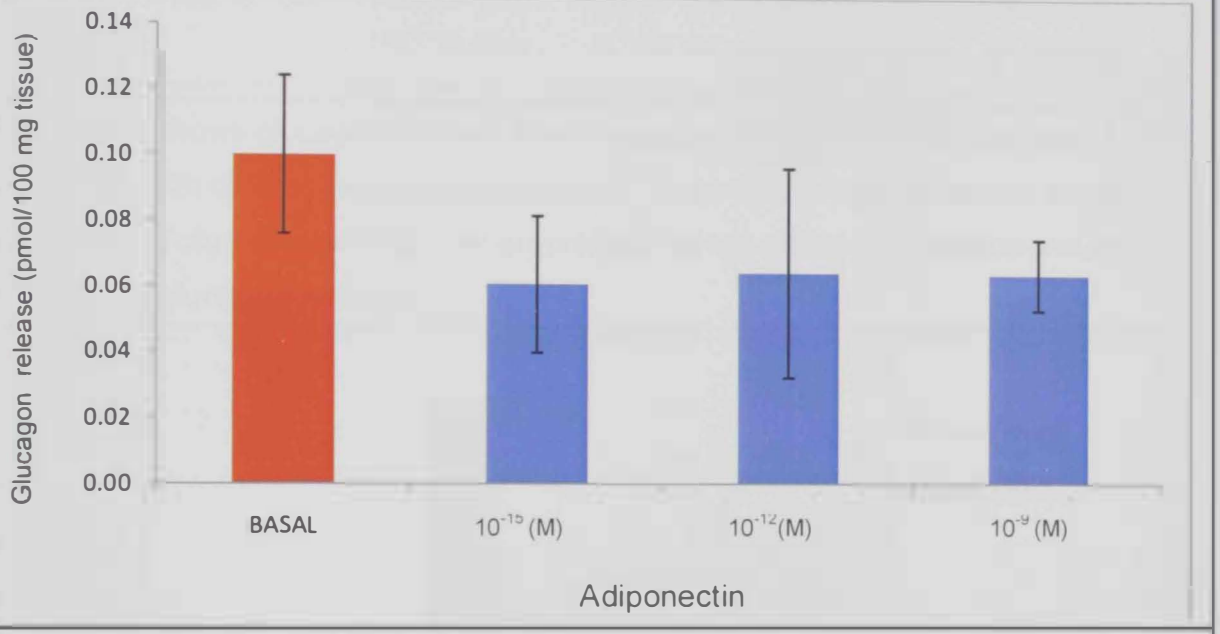



Figure (90) shows the effect of different concentrations of adiponectin on glucagon release from normal GK pancreas.

*Note that there is a slight but not a significant decrease in glucagon release from the pancreas of GK rats after treatment with adipopnectin.

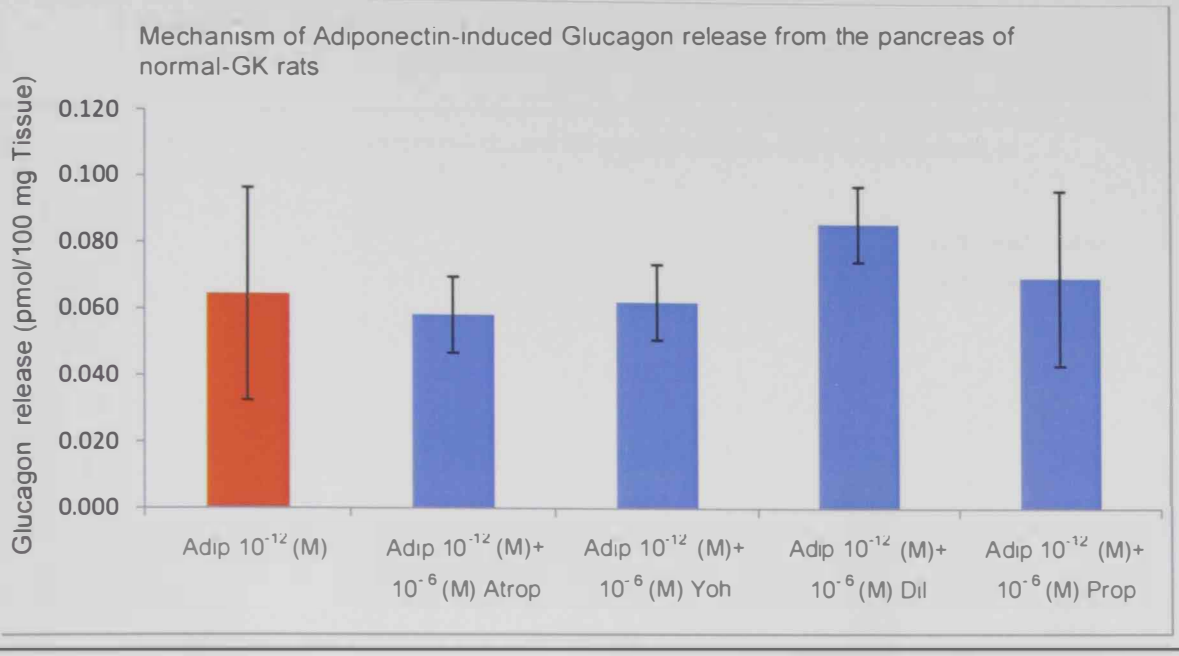



Figure (91) shows glucagon release from the pancreas of normal GK rats after incubation with different receptor antagonists. *Note that neither, atropine (atrop), yohimbine (Yoh), diltiazem (Dil) nor propranolol (prop) affects adiponectin-induced inhibition of glucagon release

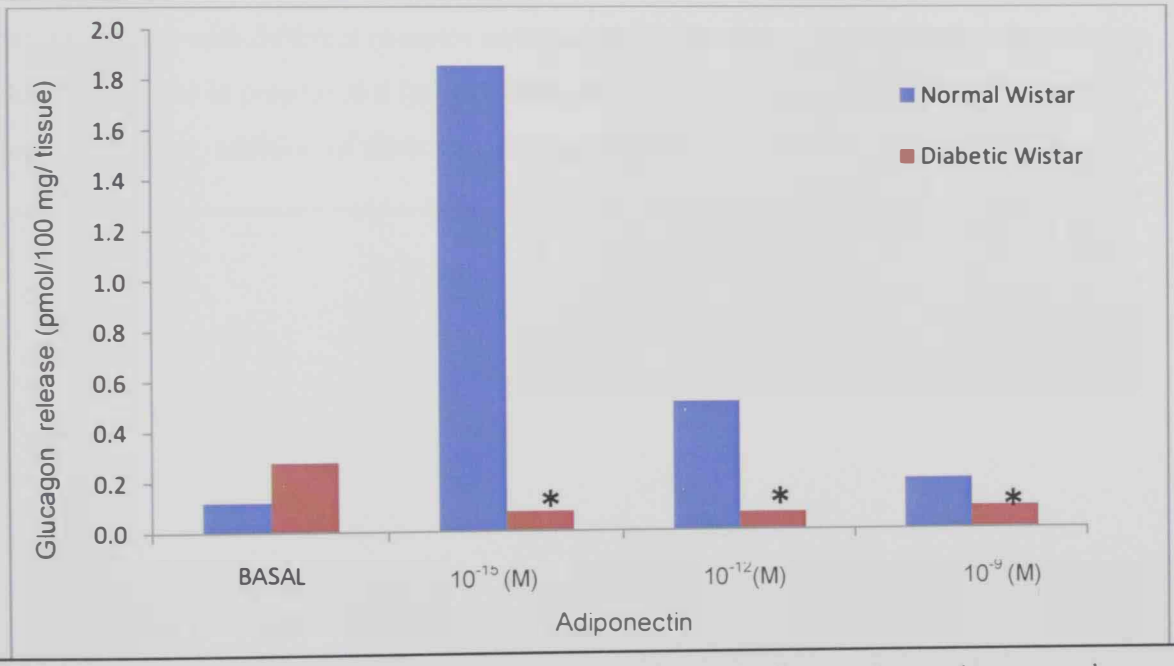



Figure (92) shows the effect of different concentrations of adiponectin on glucagon release from the pancreas of normal and diabetic Wistar rats. * Note that adiponectin caused an increase in glucagon release from the pancreas of normal rats but inhibited glucagon secretion from diabetic rats.

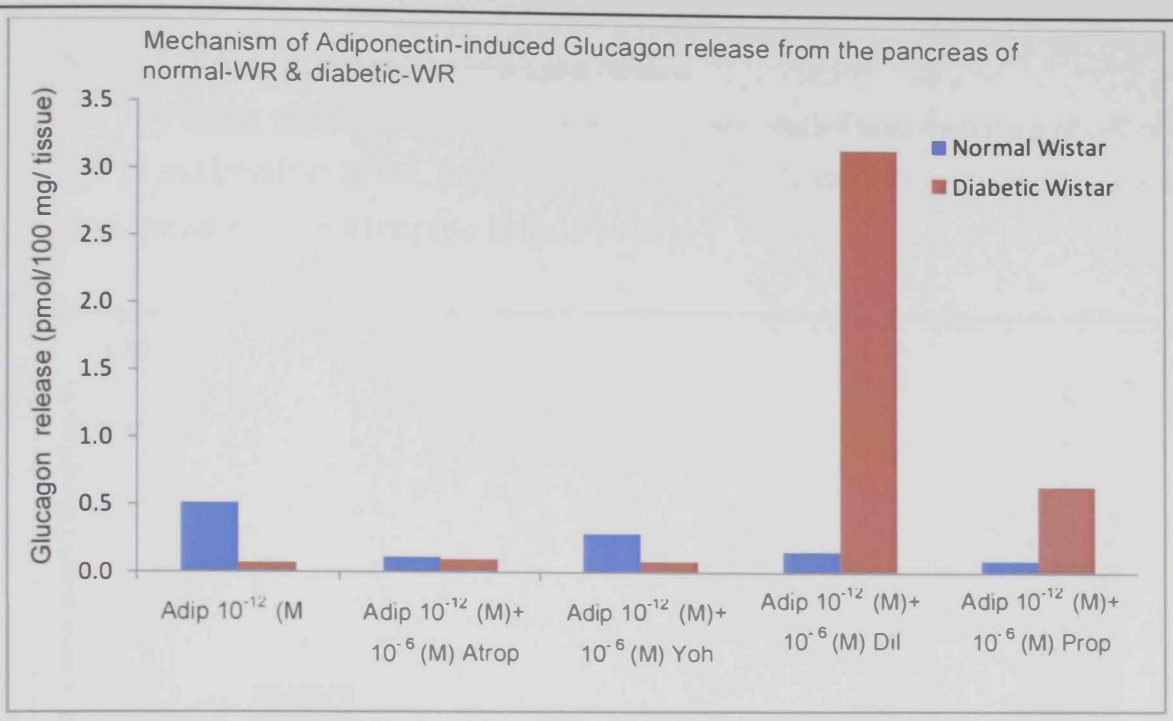



Figure (93) shows glucagon release from the pancreas of normal and diabetic Wistar rats after incubation with different receptor antagonists. *Note that neither, atropine (atrop), yohimbine (Yoh) or propranolol (prop) affects adiponectin-induced inhibition of glucagon release. However, addition of diltiazem (Dil) caused large increases in glucagon release

### VIII.2.b. Effect of visfatin on glucagon release

Visfatin stimulated glucagon release from pancreatic tissue fragments of GK rats at $10^{-6}$ M and inhibit at $10^{-9}$M. It failed to cause any significant changes in glucagon release from the pancreas of Wistar rats. (Figure 94-97)

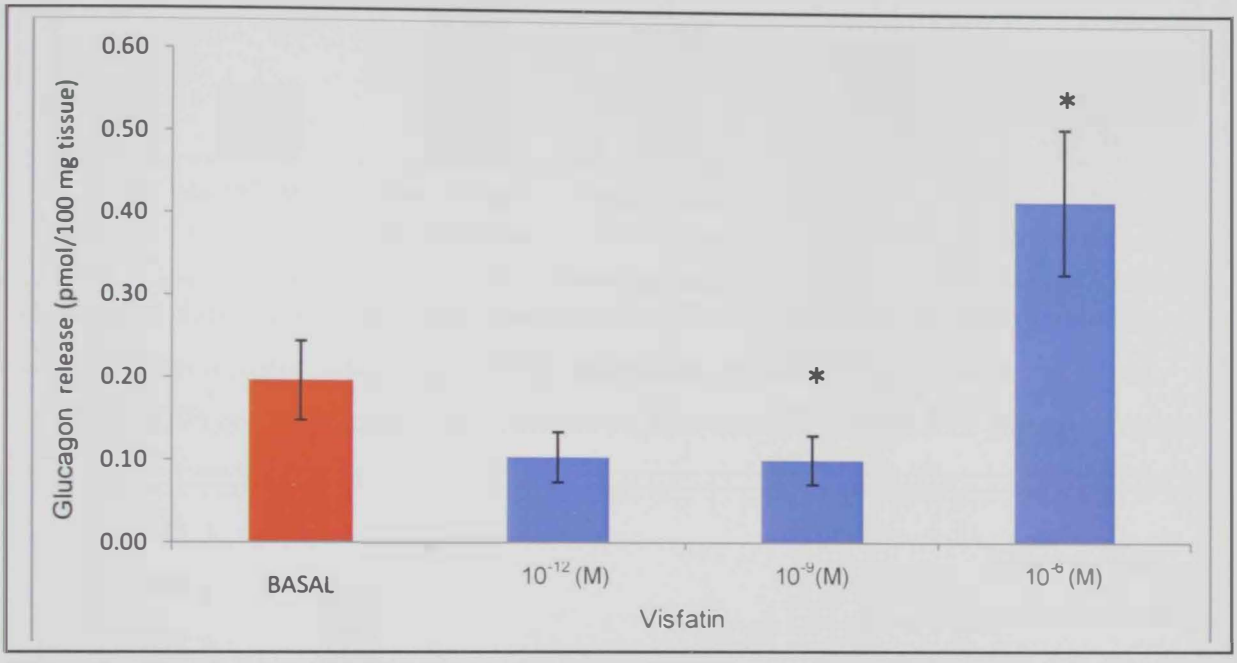



Figure (94) shows the effect of different concentrations of Visfatin on glucagon release from normal GK pancreas.

*Note that Visfatin shows a biphasic effect, causing a significant decrease in glucagon release at low concentration and a significant increase at high concentration.

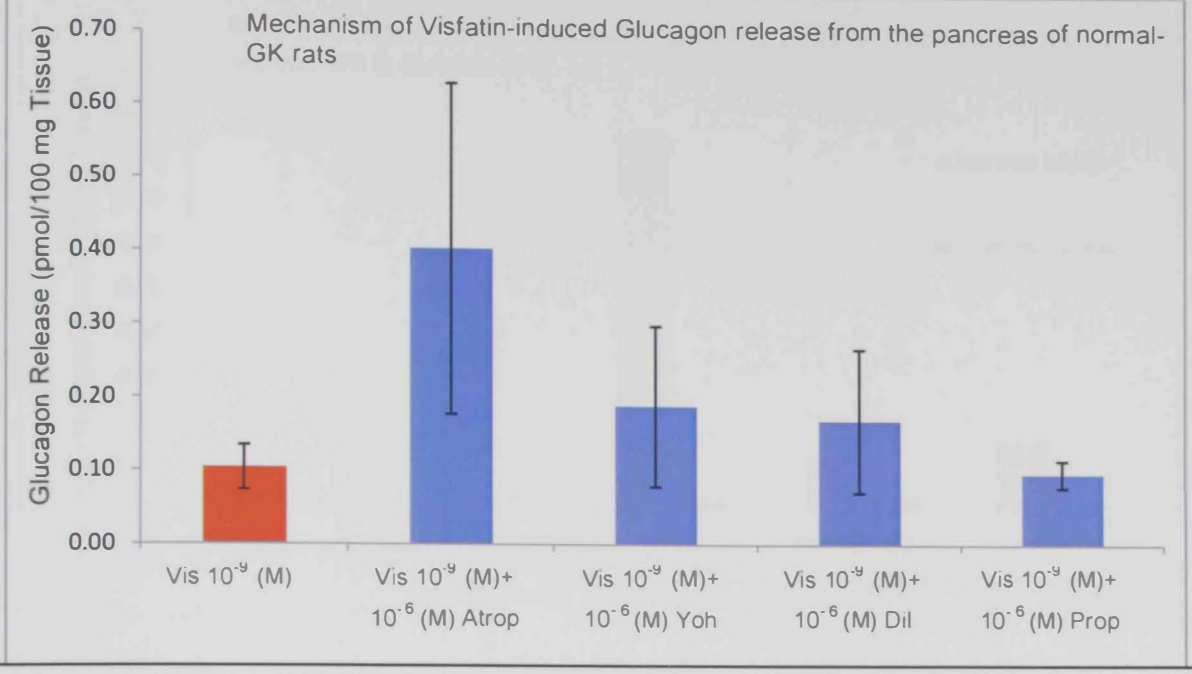



Figure (95) shows glucagon release from the pancreas of normal GK rats after incubation with different receptor antagonists. *Note that neither, atropine (atrop), yohimbine (Yoh), diltiazem (Dil) nor propranolol (prop) affects visfatin-induced inhibition of glucagon release

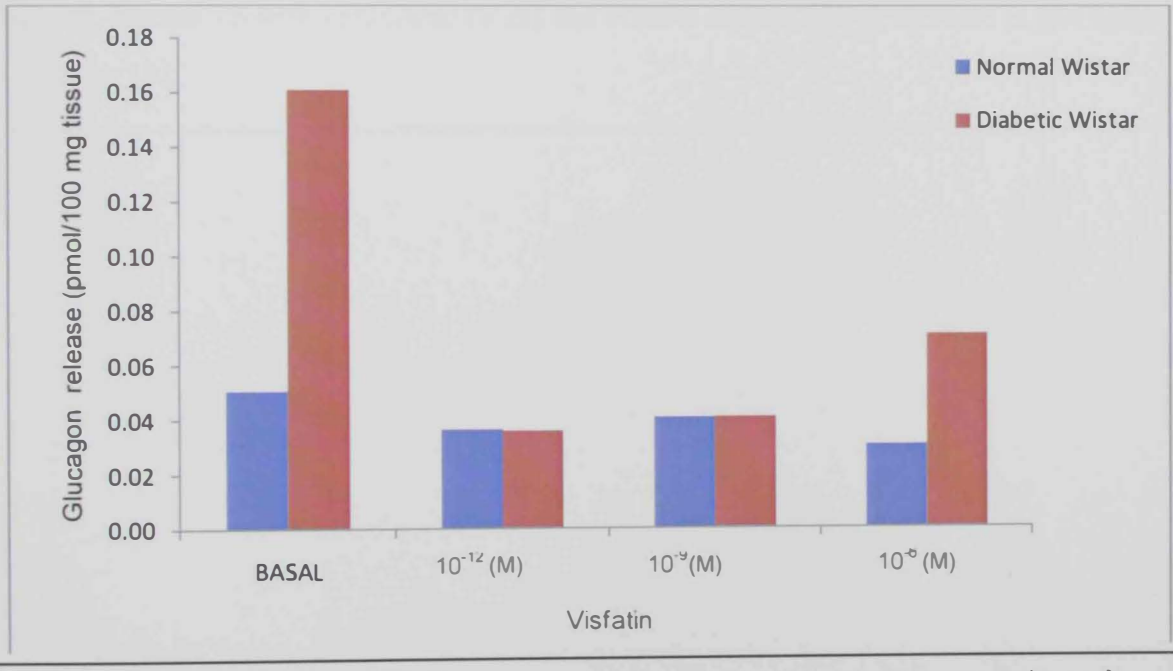



Figure (96) shows the effect of different concentrations of visfatin on glucagon release from the pancreas of normal and diabetic Wistar rats. Visfatin has no effect of glucagon secretion in normal rats but appear to inhibit glucagon release from the pancreas of diabetic rats (n = 6).

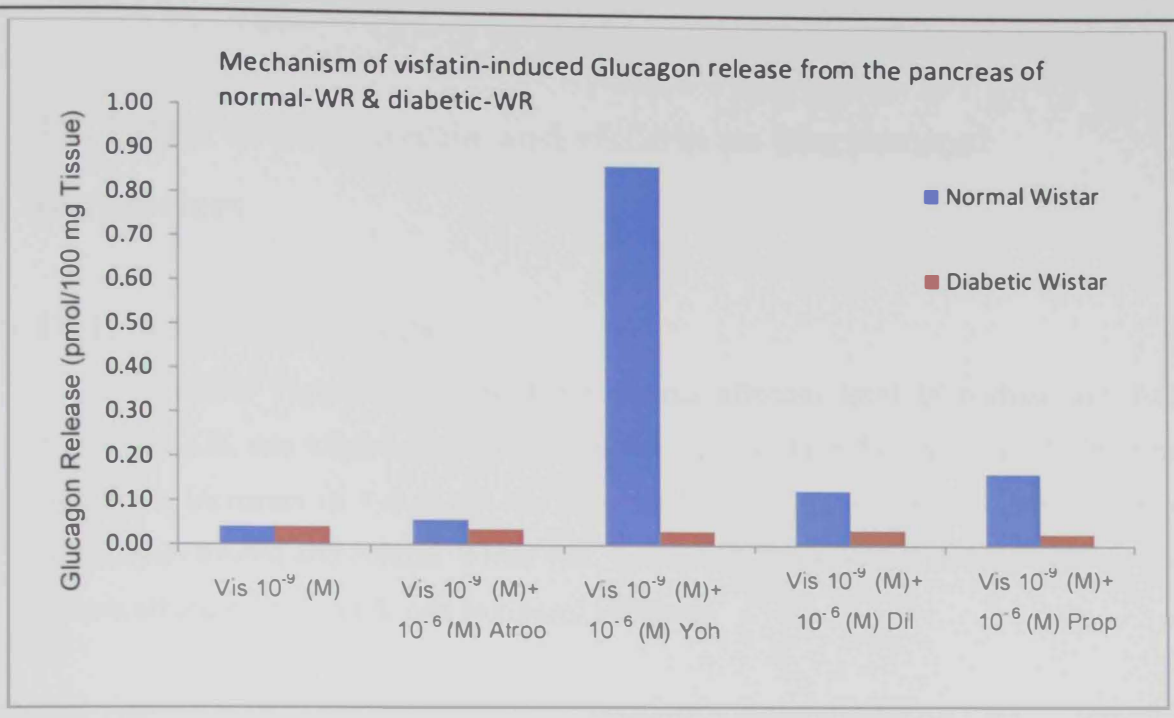



Figure (97) shows glucagon release from the pancreas of normal and diabetic Wistar rats after incubation with different receptor antagonists. *Note that neither, atropine (atrop), diltiazem (Dil) nor propranolol (prop) affects visfatin-induced inhibition of glucagon release. However, incubation with yohimbine (Yoh) and visfatin caused a large increase in glucagon release. (n = 6).

# IX. Effect of adiponectin and visfatin on biochemical parameters

## IX.1. Liver function tests

Albumin: Figures (98-100) shows plasma albumin level in normal and diabetic Wistar and GK rats treated with either adiponectin or visfatin for 14 weeks. Visfatin cause significant increases ($p < 0.05$) in the albumin level of diabetic Wistar rats compared to adiponectin-treated and control Wistar rats. In contrast, adiponectin induce large increase in plasma albumin level in GK rats compared to control

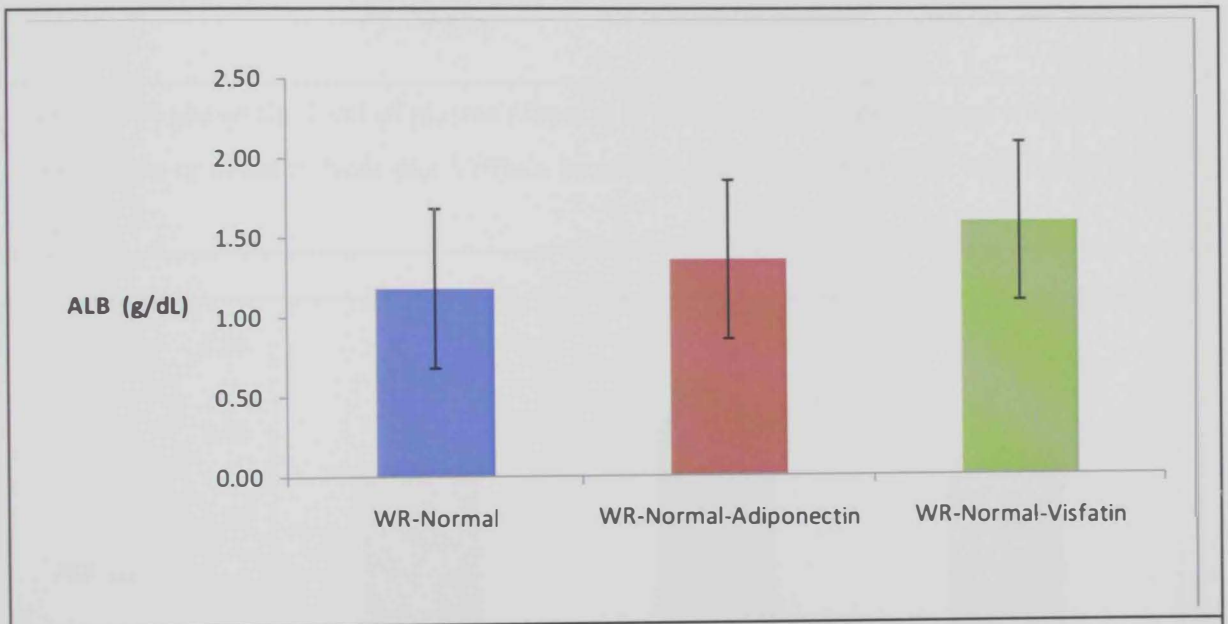



Figure (98) shows the level of plasma albumin in normal Wistar rats treated with either adiponectin or visfatin. Neither visfatin nor adiponectin has effect on plasma albumin level.

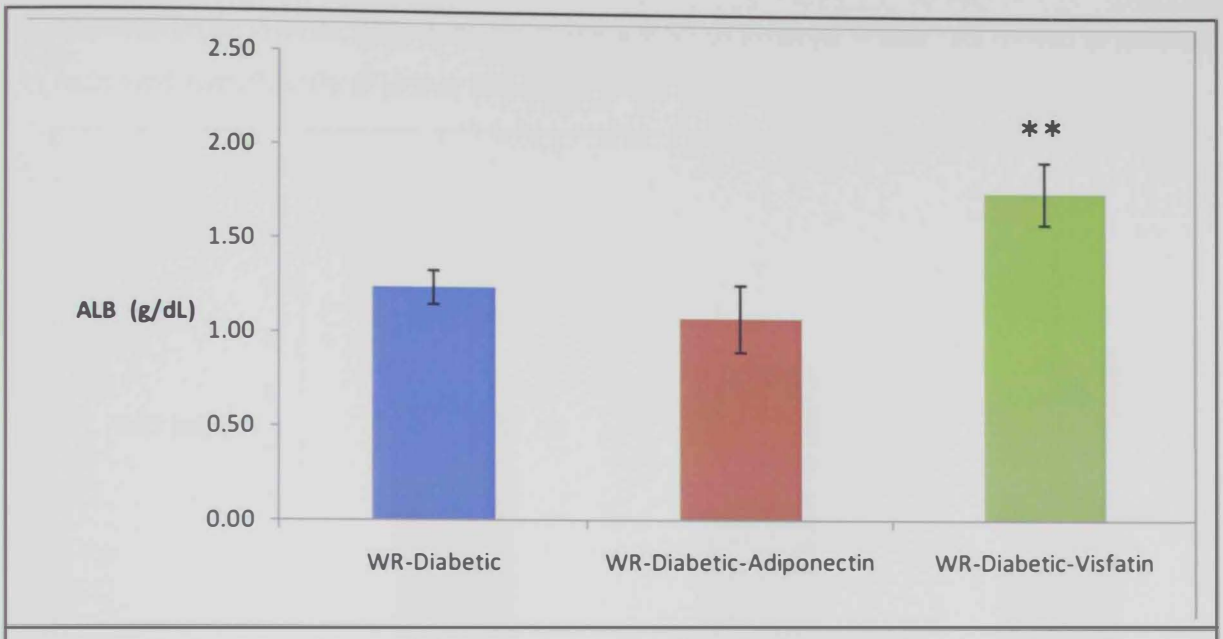



Figure (99) shows the level of plasma albumin in diabetic Wistar rats treated with either adiponectin or visfatin. Note that Visfatin increased plasma albumin level significantly ($P < 0.05$)

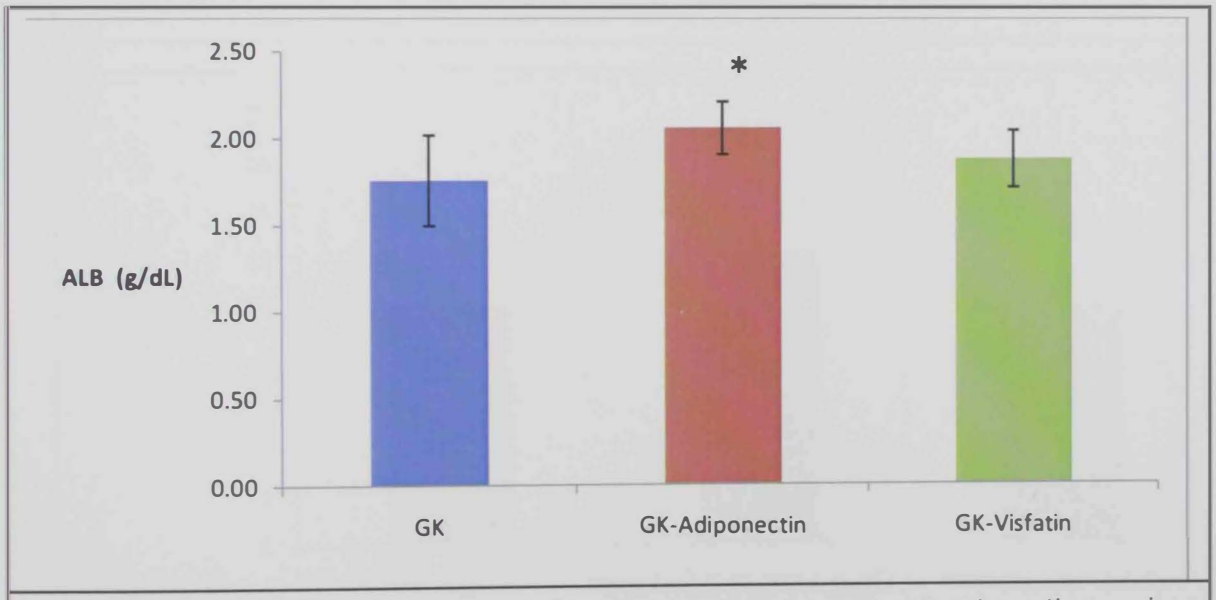



Figure (100) shows the level of plasma albumin in GK rats treated with either adiponectin or visfatin. Note that adiponectin caused a significant ($P < 0.05$) increase in plasma albumin level

The level of GGT was not altered in GK and normal Wistar rats treated with either adiponectin or visfatin. However, the plasma level of diabetic Wistar rats treated adiponectin increased significantly (Figures 101-103).

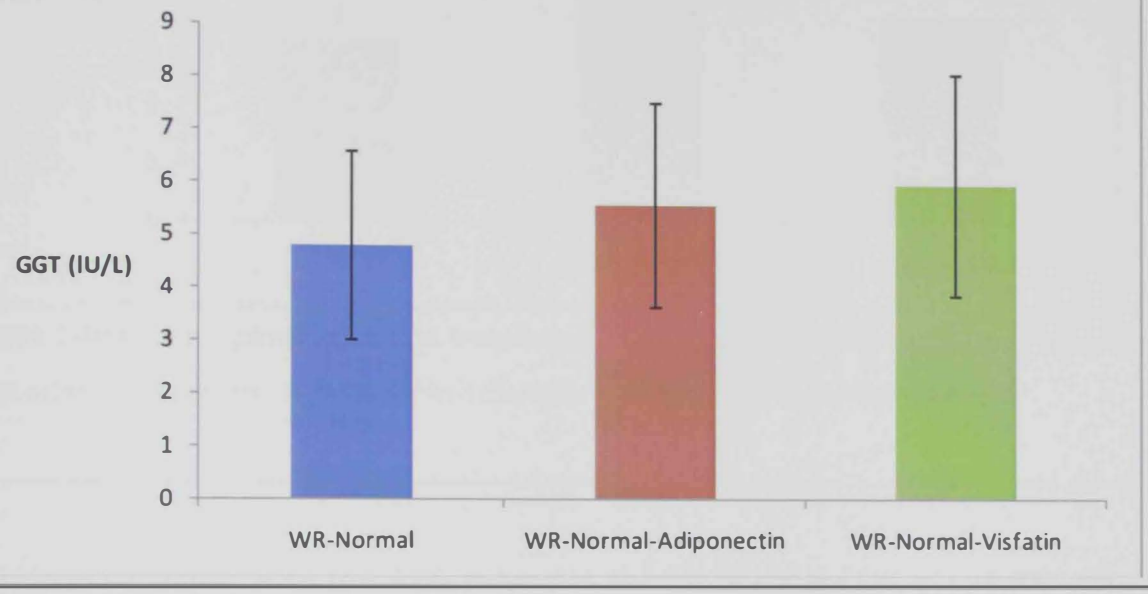



Figure (101) shows the level of gammaglutamyl transferase (GGT) plasma level in normal Wistar rats. No difference was observed in the three groups.

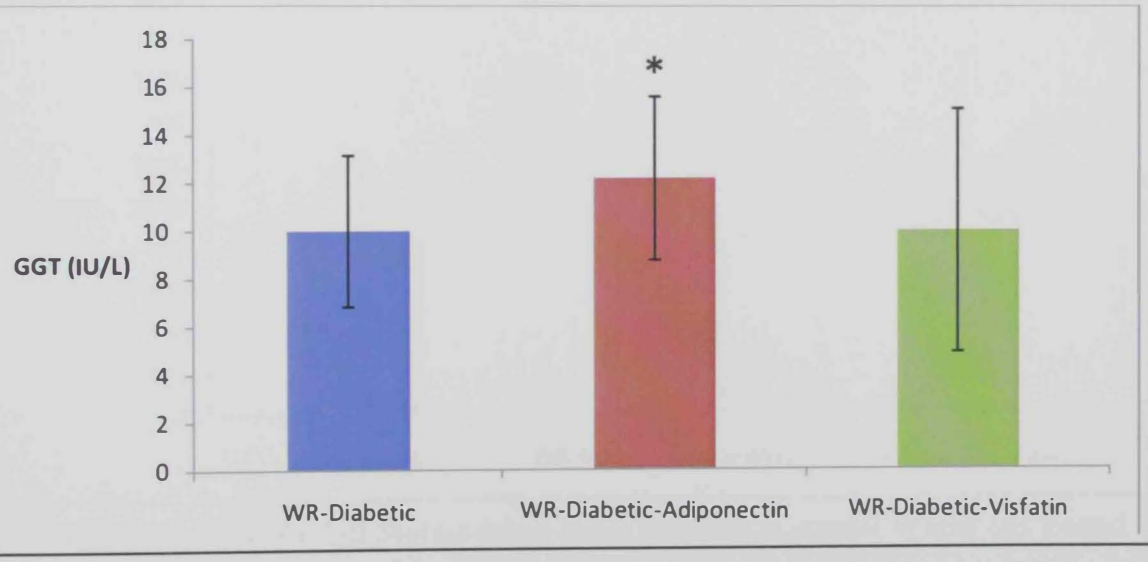



Figure (102) shows gammaglutamyl transferase (GGT) plasma level in normal Wistar rats treated with either adiponectin or visfatin. Note that Wistar diabetic rats treated with adiponectin show significant ($p < 0.05$) increase in GGT plasma level.

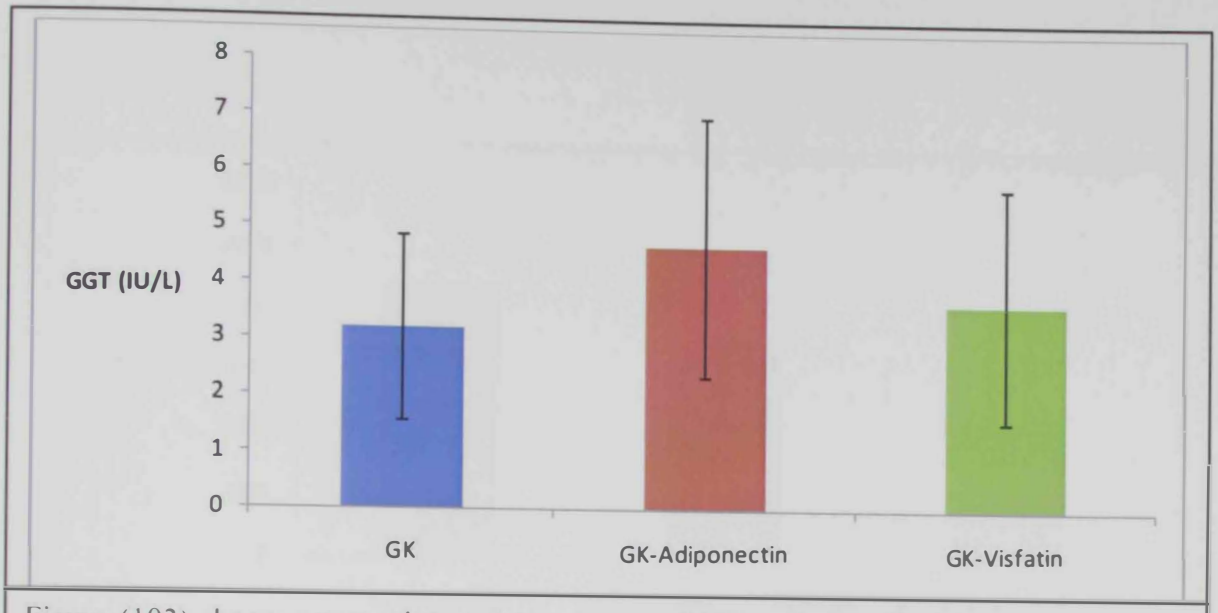



Figure (103) shows gammaglutamyl transferase (GGT) plasma level in GK rats treated with either adiponectin or visfatin. No difference was observed in the three groups

LDH level was significantly ($p < 0.05$) reduced in diabetic Wistar and GK rats treated with visfatin (Figures 104-105).

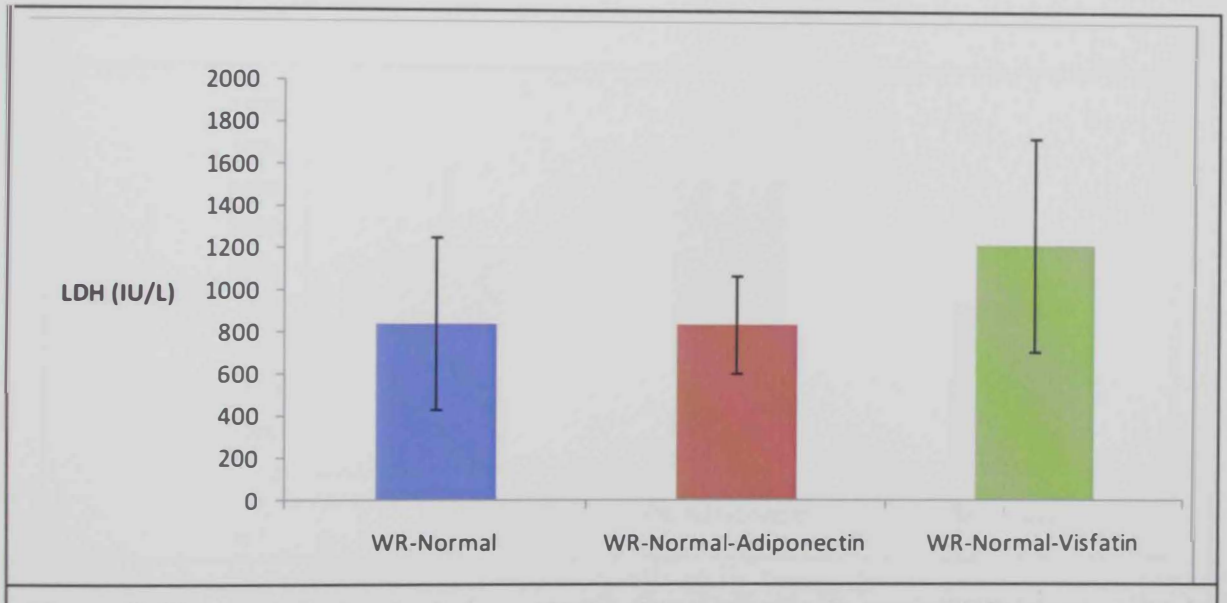



Figure (104) shows the level of plasma lactic dehydrogenase in normal Wistar rats treated with either adiponectin or visfatin. There was no significant difference between the 3 groups.

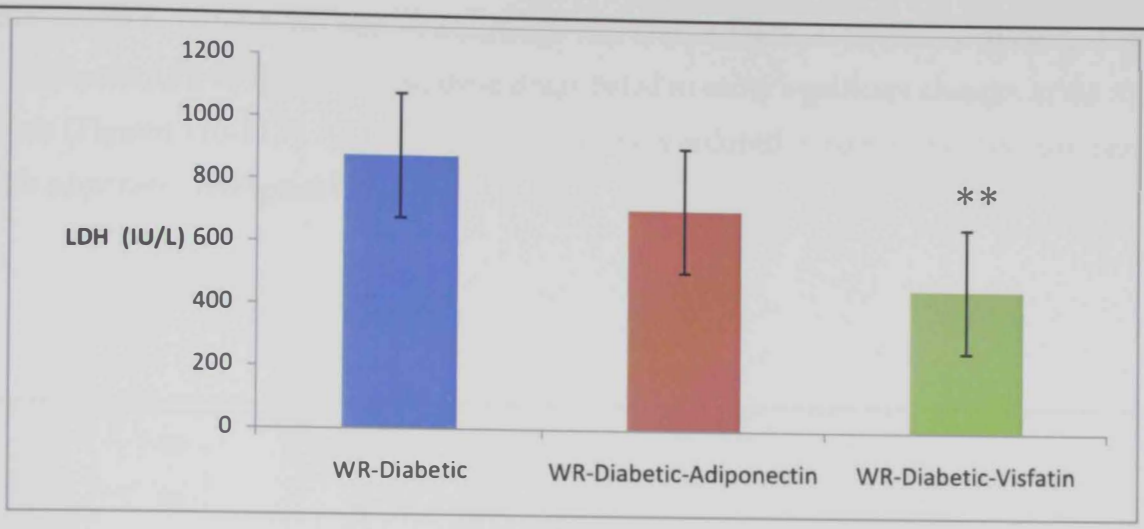



Figure (105) shows the level of plasma lactic dehydrogenase in diabetic Wistar rats treated with either adiponectin or visfatin. Note that diabetic rats treated with visfatin show significant ($p < 0.05$) decrease in lactic dehydrogenase plasma level

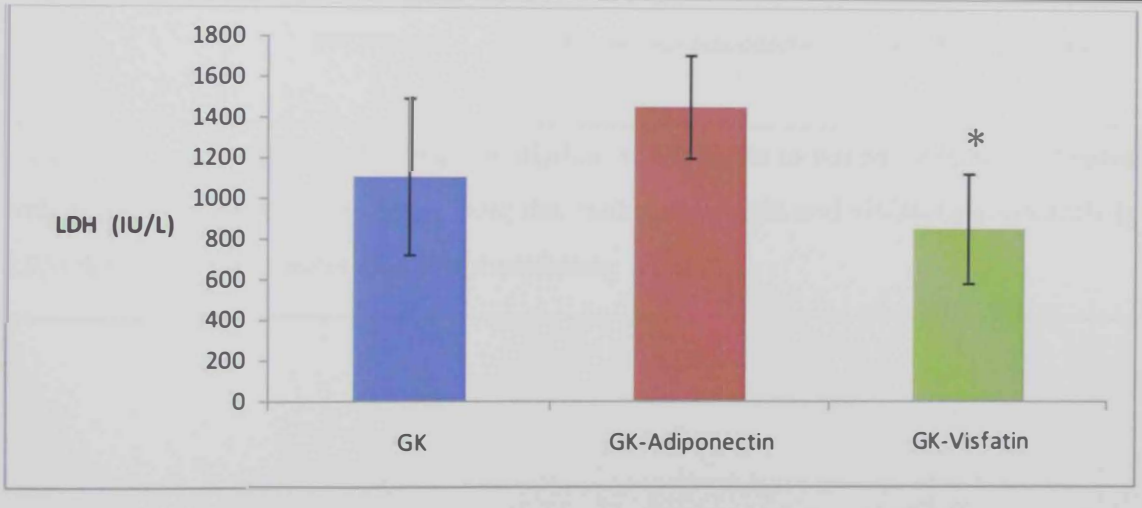



Figure (106) shows the level of plasma lactic dehydrogenase in GK rats treated with either adiponectin or visfatin. Note that GK rats treated with visfatin show a significant ($p < 0.05$) decrease in lactic dehydrogenase plasma level.

ALP level was significantly reduced in normal Wistar and GK rats treated with either adiponectin or visfatin. No significant change was seen in the level of ALP in diabetic Wistar rats (Figures 107-109). In contrast, these drugs failed to cause significant changes in the ALT levels (Figures 110-112). AST level was significantly reduced in normal and GK rats treated with adiponection (Figures113 -115).

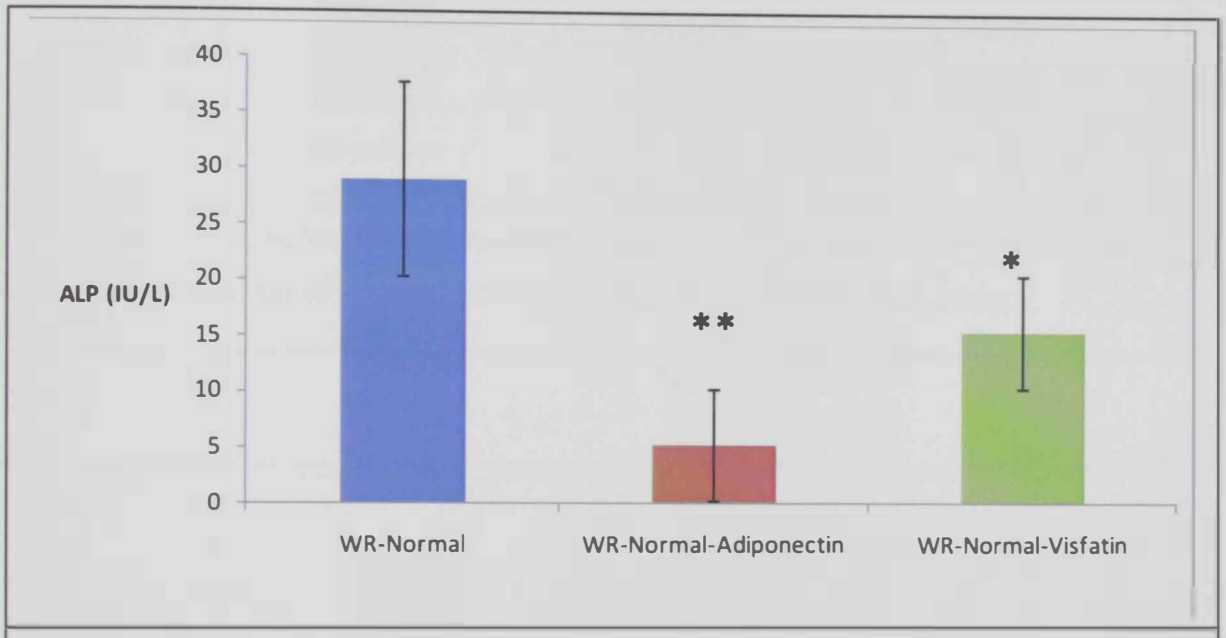



Figure (107) shows the level of plasma alkaline phosphatase in normal Wistar rats treated with either adiponectin or visfatin. Note that both adiponectin and visfatin significantly ($p < 0.05$) decreased the plasma alkaline phosphatase level

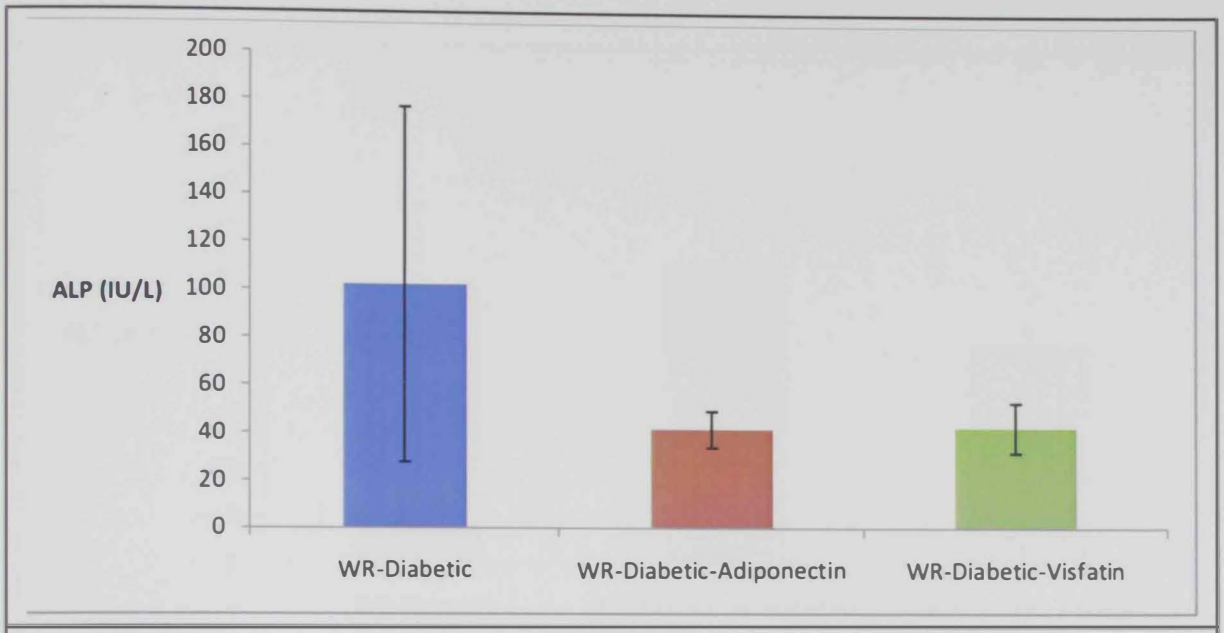



Figure (108) shows the level of plasma alkaline phosphatase in diabetic Wistar rats treated with either adiponectin or visfatin. Note that ALP is lower in the treated group.

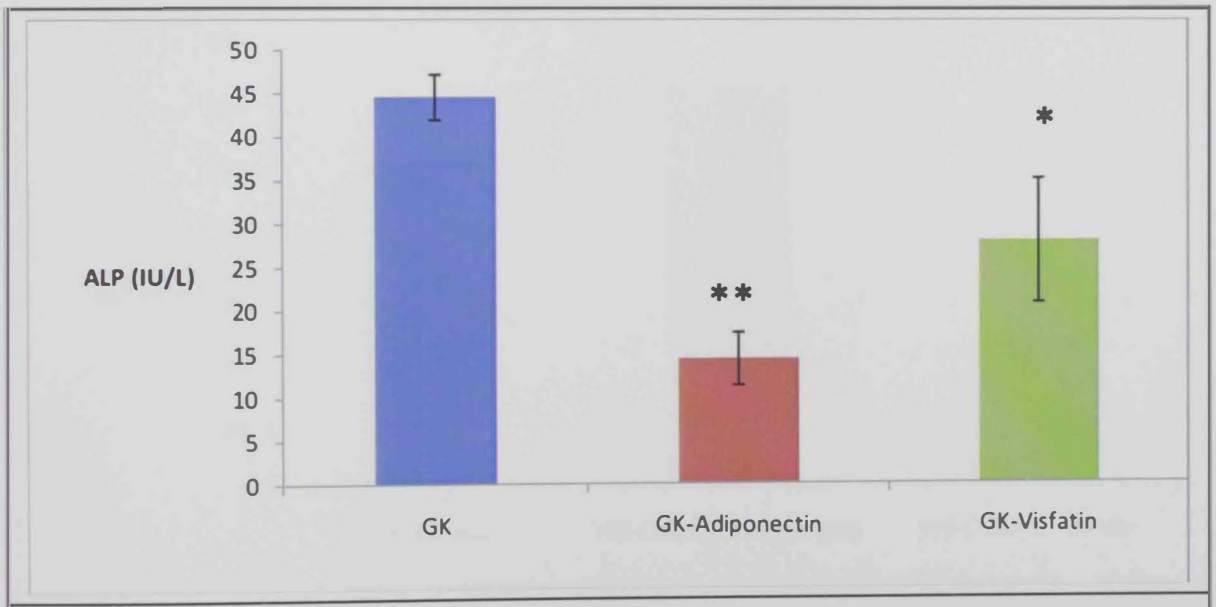



Figure (109) shows the level of plasma alkaline phosphatase in GK rats treated with either adiponectin or visfatin. Note that either adiponectin or visfatin significantly ($p < 0.05$) caused a decrease in plasma alkaline phosphatase level.

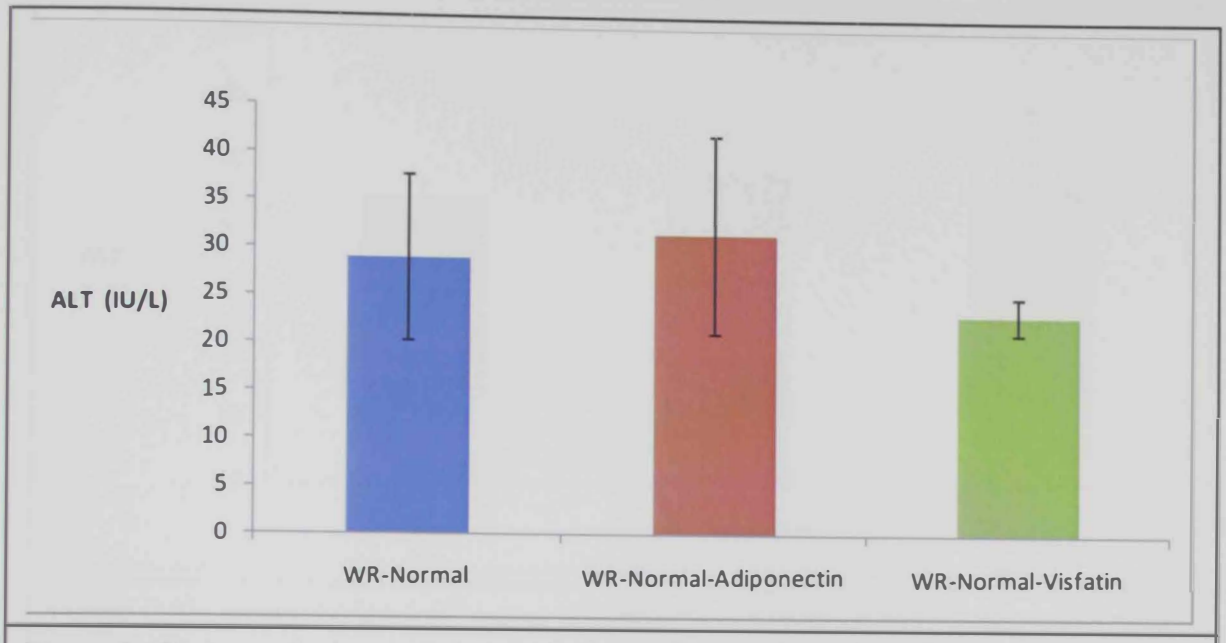



Figure (110) shows the level of plasma alanine aminotransferase in normal Wistar rats treated with either adiponectin or visfatin. No significant difference was seen in the three groups.

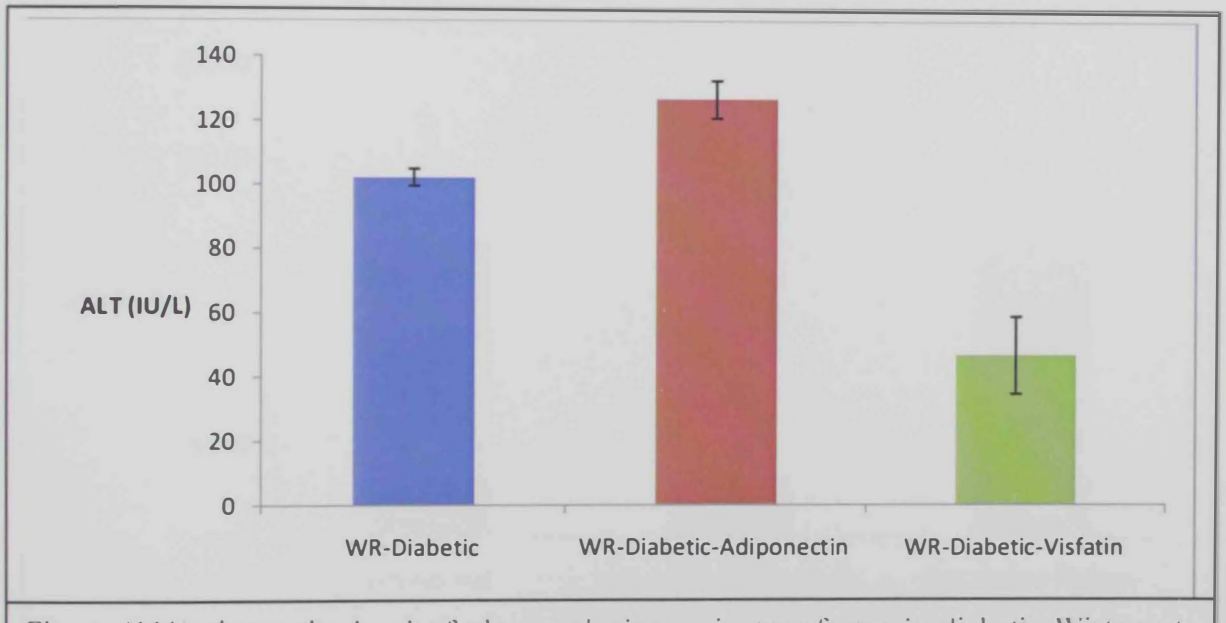



Figure (111) shows the level of plasma alanine aminotransferase in diabetic Wistar rats treated with either adiponectin or visfatin. There was a slight but not significant decrease in ALT level after treatment with visfatin.

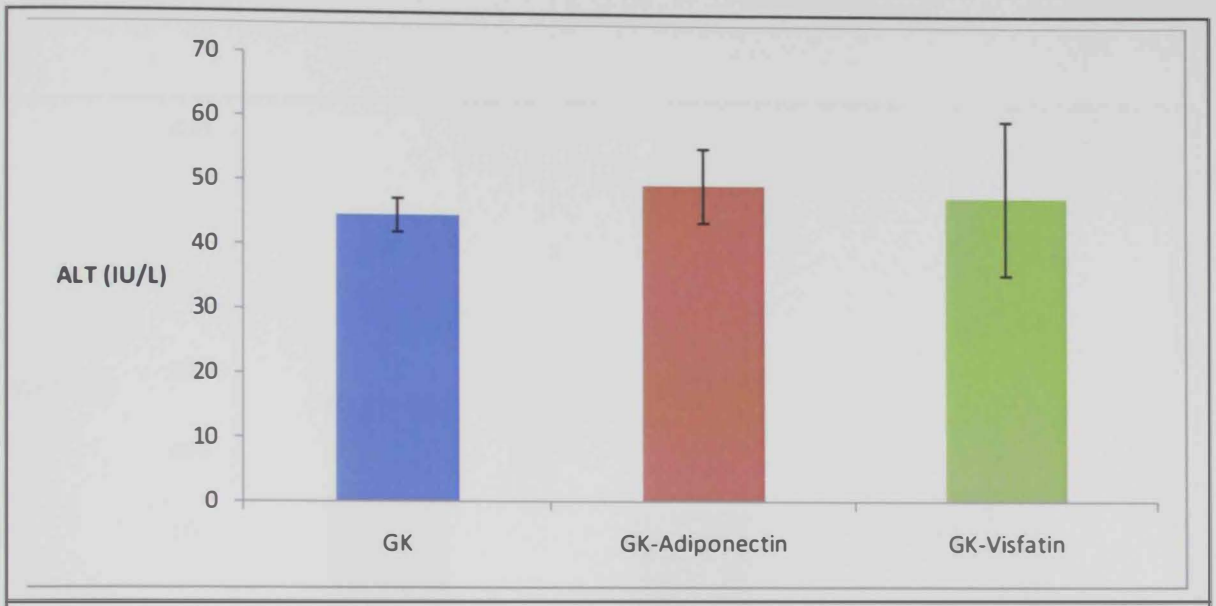



Figure (112) shows the level of plasma alanine aminotransferase in GK rats treated with either adiponectin or visfatin. No significant difference was seen in the three groups.

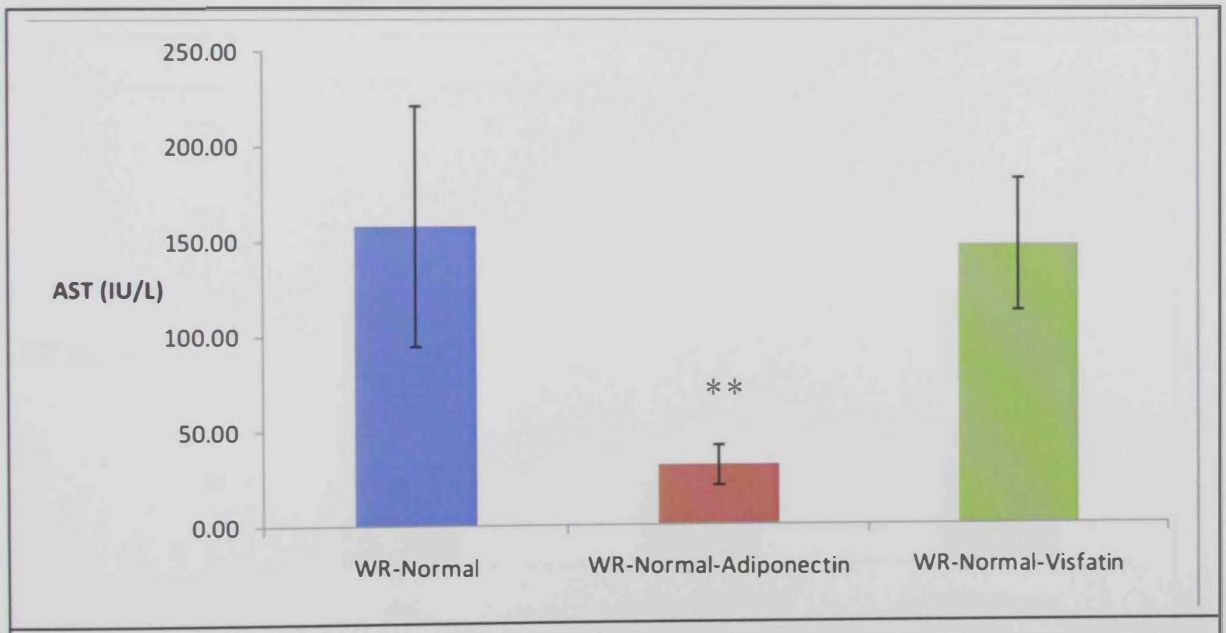



Figure (113) shows aspartate aminotransferase plasma level in normal Wistar rats treated with either adiponectin or visfatin. Note that adiponectin-treated rats displayed significant ($p < 0.05$) reduction in plasma AST level.

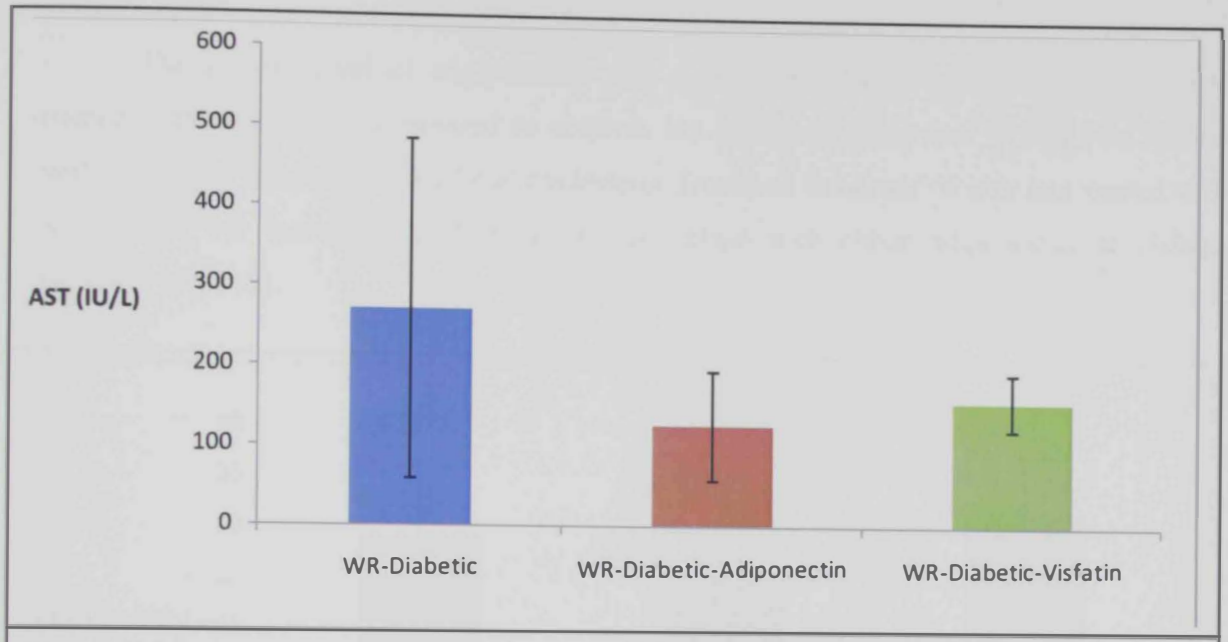



Figure (114) shows aspartate aminotransferase plasma level in diabetic Wistar rats treated with either adiponectin or visfatin. Note that rats treated with either adiponectin or visfatin showed a slight but not significant decrease in plasma AST level.

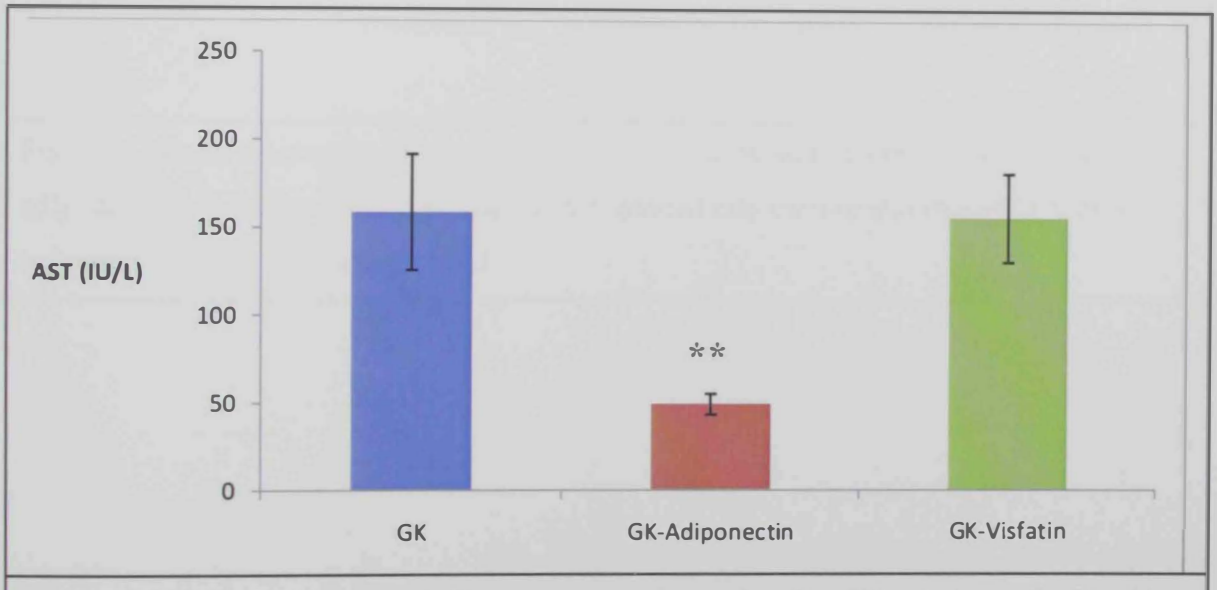



Figure (115) shows aspartate aminotransferase plasma level in GK rats treated with either adiponectin or visfatin. Note that adiponectin-treated rats displayed significant ($p < 0.05$) reduction in plasma AST level.

## IX.2. Lipids

The plasma level of triglycerides was significantly lower in diabetic Wistar rats treated with adiponectin compared to controls but increased in normal Wistar rats treated with visfatin (Figures119-121). Total cholesterol decreased in normal Wistar rats treated with adiponectin but increased in diabetic Wistar treated with either adiponectin or visfatin (Figures 116-118).

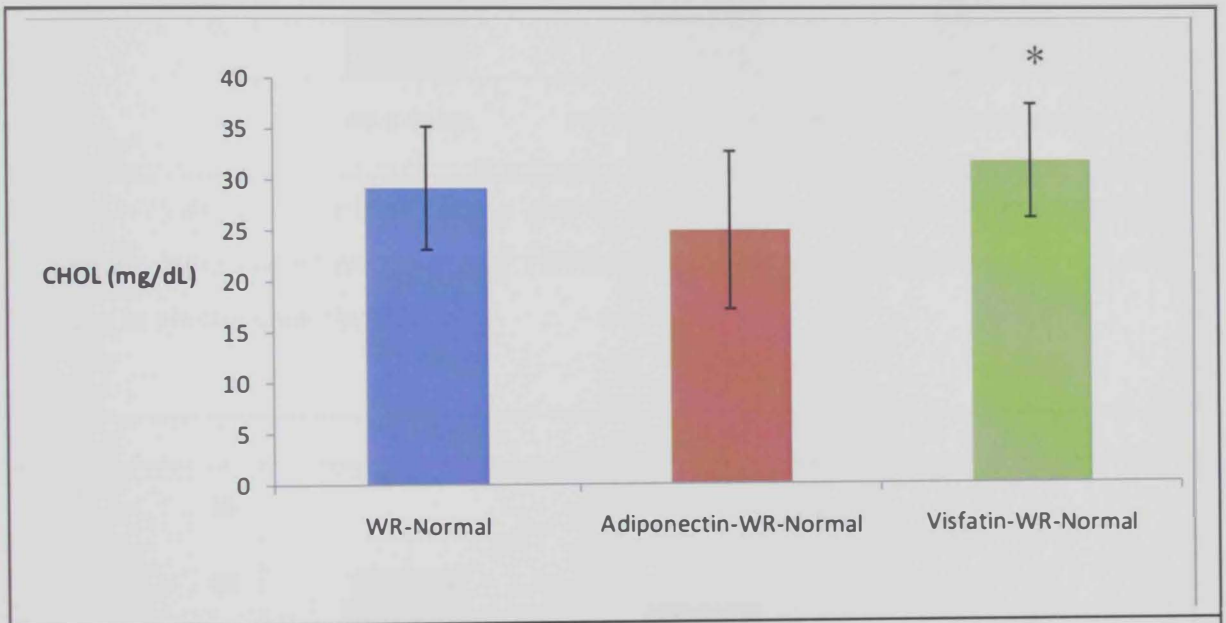



Figure (116) shows cholesterol plasma levels in normal Wistar rats treated with either adiponectin or visfatin. Note that adiponectin- treated rats showed significant ($p < 0.05$) reduction in plasma cholesterol level.

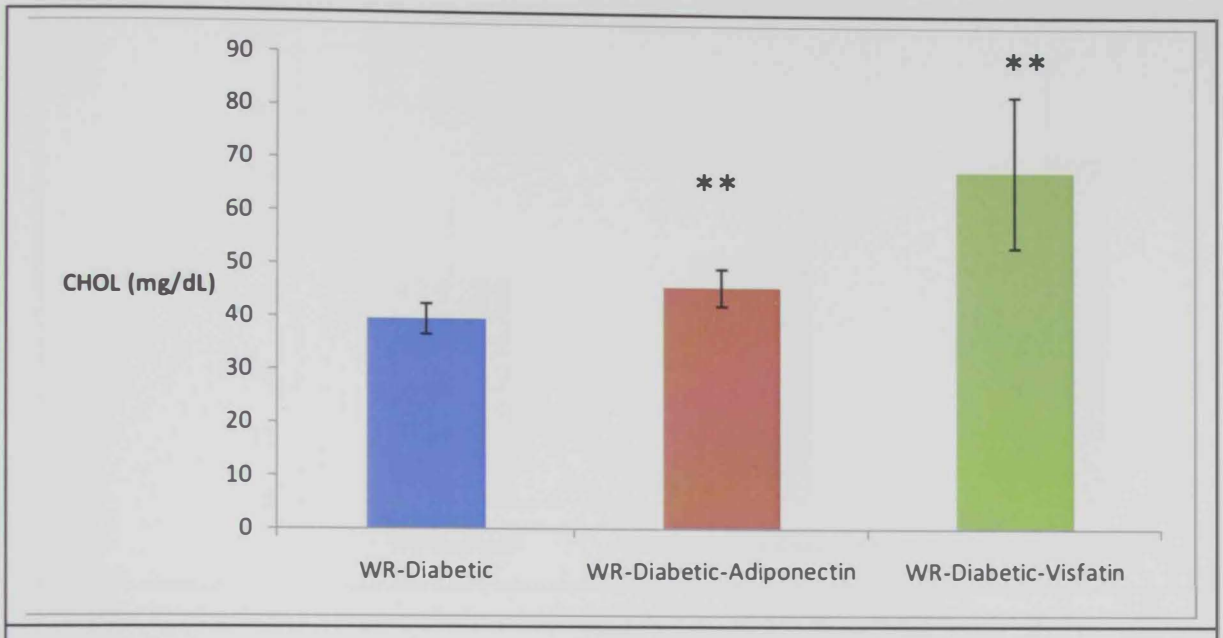



Figure (117) shows cholesterol plasma level in diabetic rats treated with either adiponectin or visfatin. Note that adiponectin- and visfatin-treated rats showed significant ($p < 0.005$) increase in plasma cholesterol level.

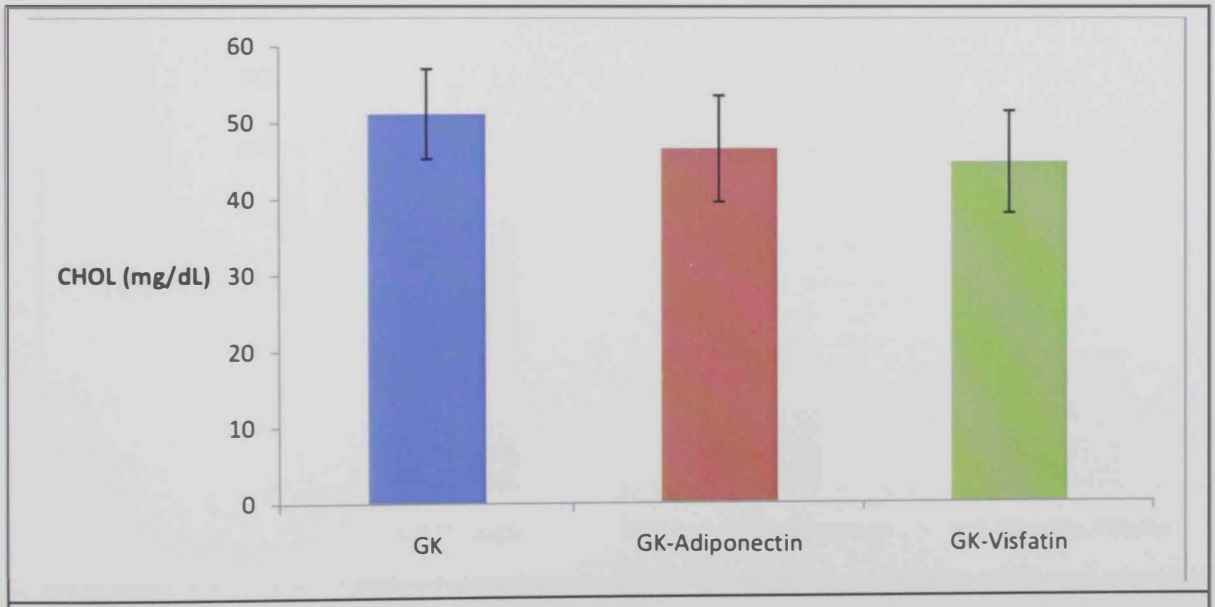



Figure (118) shows cholesterol plasma level in GK rats treated with either adiponectin or visfatin. Neither drug has any effect of plasma cholesterol level.

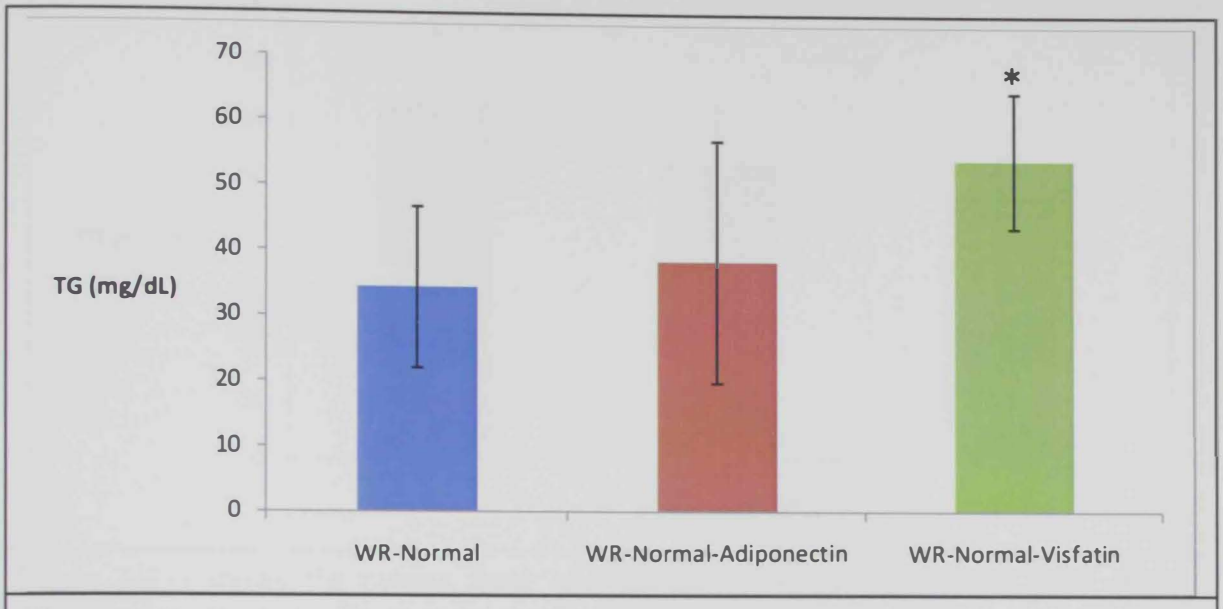



Figure (119) shows the plasma level of triglycerides in normal Wistar rats treated with either adiponectin or visfatin. Wistar normal rats treated with visfatin show significant ($p < 0.05$) increase in plasma triglycerides level.

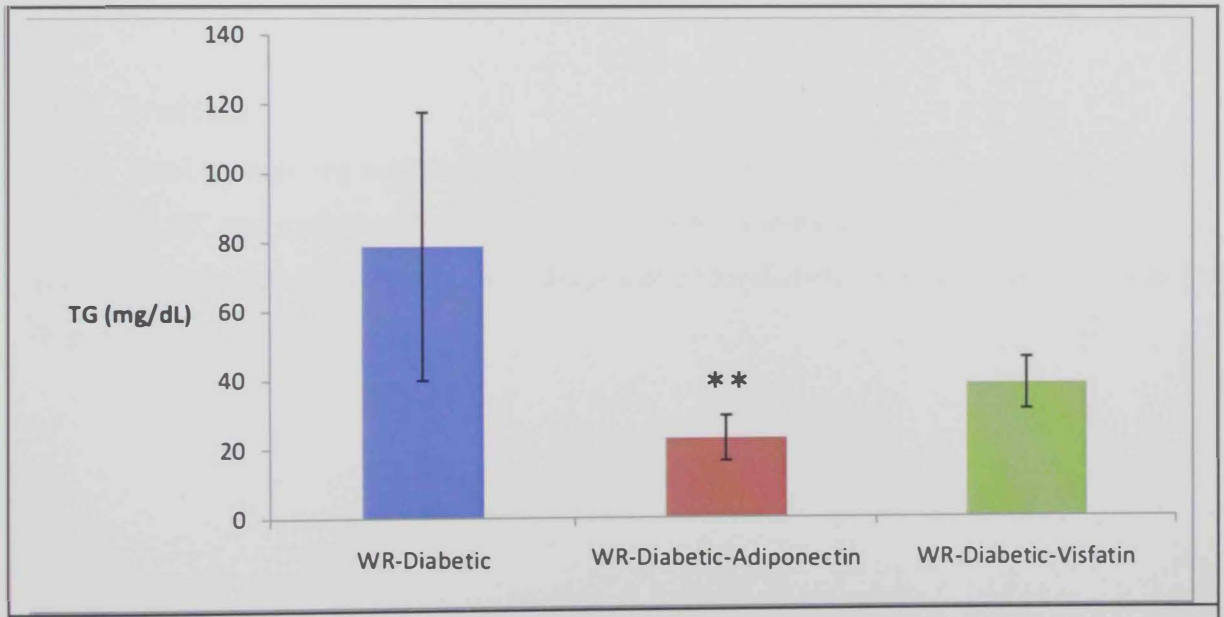



Figure (120) shows the plasma level of triglycerides in diabetic Wistar rats treated with either adiponectin or visfatin. Note that diabetic Wistar rats treated with adiponectin show significant ($p < 0.005$) decrease in plasma triglycerides level.

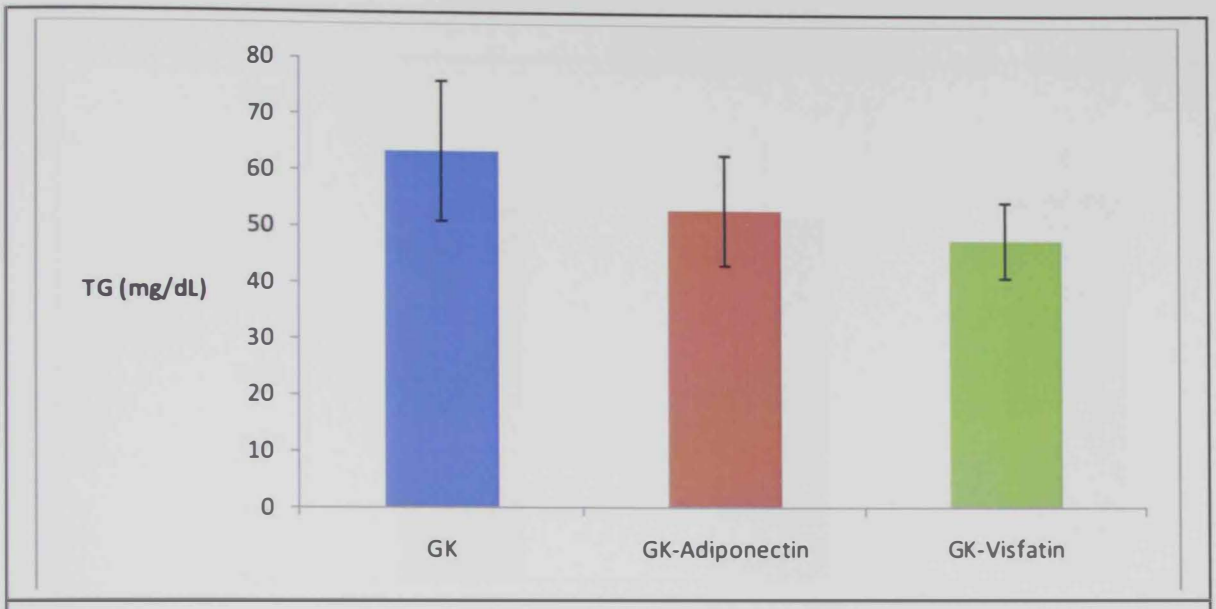



Figure (121) shows the plasma level of triglycerides in GK rats treated with either adiponectin or visfatin. There is no significant difference between the 3 groups although there was a trend towards a lower TG in treated rats.

## IX.3. Proteins

Total protein was not modified by the administration of either adiponectin or visfatin except for GK rats treated with adiponectin. Similarly, plasma amylase was not significantly altered after the administration of these drugs except for diabetic Wistar treated with visfatin (Figures 122-124).

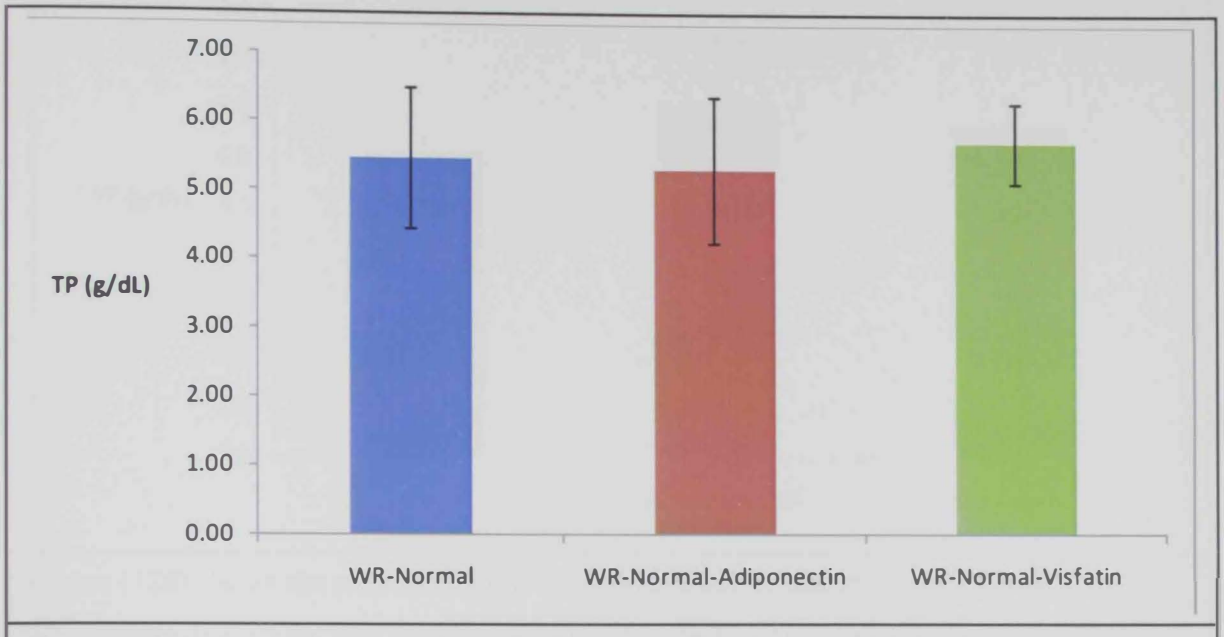



Figure (122) shows the plasma level of total protein in normal Wistar rats treated with either adiponectin or visfatin. There was no significant difference between the 3 groups.

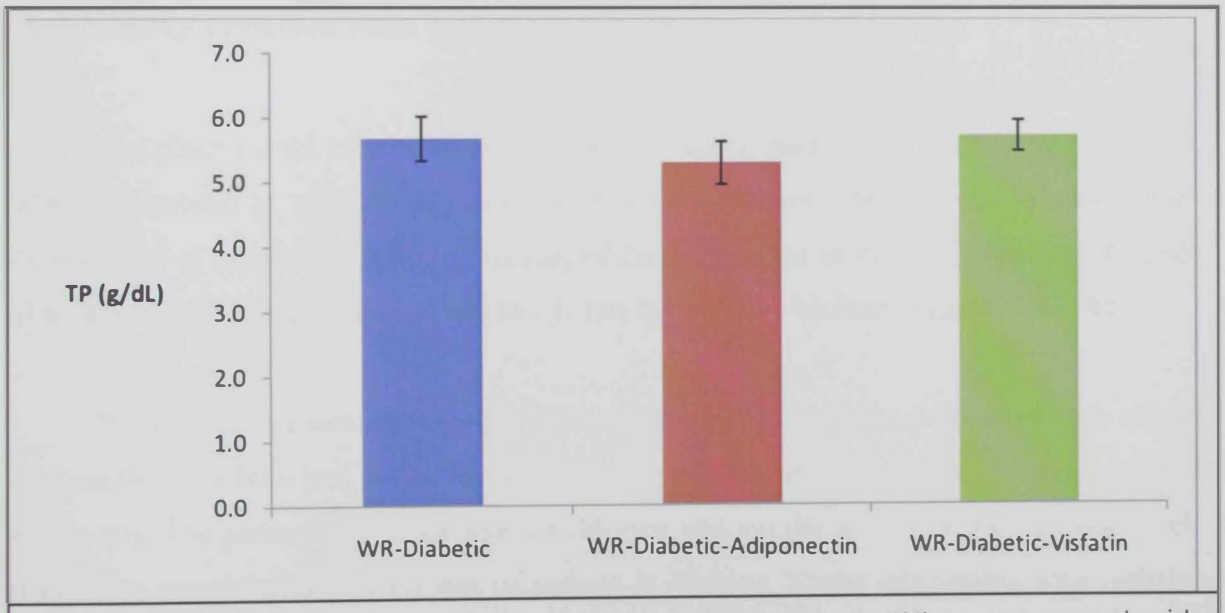



Figure (123) shows the plasma level of total protein in diabetic Wistar rats treated with either adiponectin or visfatin. There was no significant difference between the 3 groups.

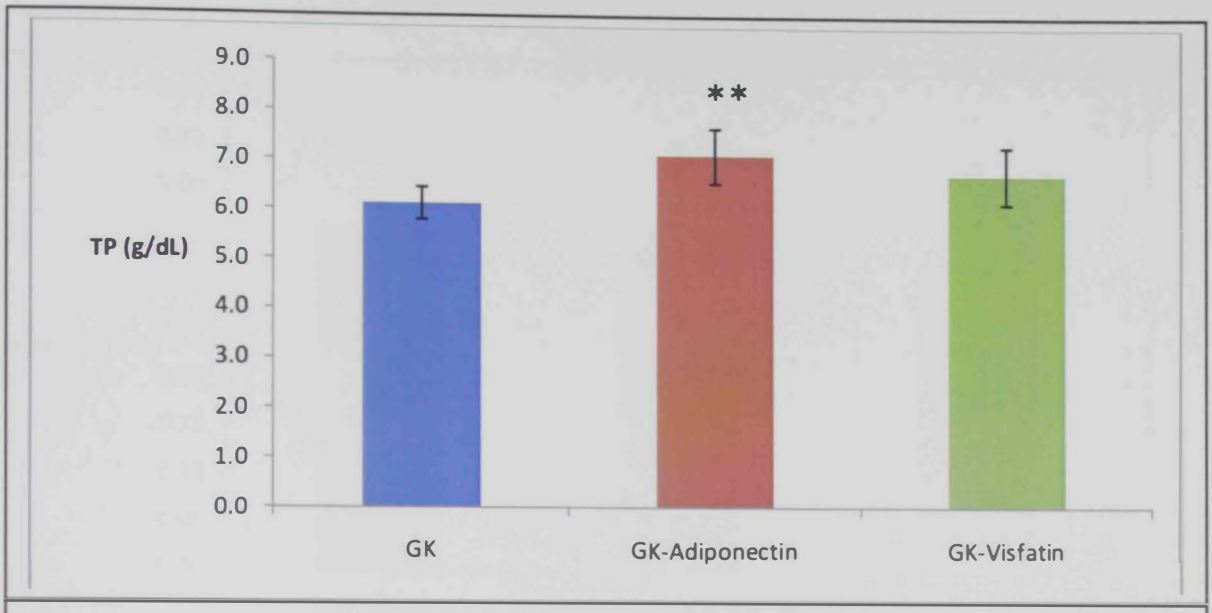



Figure (124) shows the plasma level of total protein in GK rats treated with either adiponectin or visfatin. GK rats treated with adiponectin show significant ($p < 0.005$) increase in plasma total protein level.

## IX.4.Kidney function tests and electrolytes

The plasma level of creatinine was not particularly altered after the administration of either adiponectin or visfatin to either GK, normal or diabetic Wistar rats. However, the plasma level of blood urea nitrogen was significantly reduced in normal Wistar rats treated with either adiponectin or visfatin and in GK rats treated with visfatin (Figures 125-130).

Plasma sodium was significantly increased in diabetic Wistar rats treated with either adiponectin or visfatin and in GK rats treated with adiponectin but was not altered in normal Wistar rats. The pattern of plasma level of chloride was similar to that of sodium. The level of chloride rose in tandem with that of sodium in diabetic Wistar rats treated with visfatin (Figures 131-136). The plasma level of phosphorus rose in diabetic Wistar rats treated with either adiponectin or visfatin and in GK rats treated with adiponectin (Figures 137-139). Total osmolality was higher in diabetic Wistar rats treated with visfatin and GK rats treated with adiponectin. No other changes were observed (Figures 140-142).

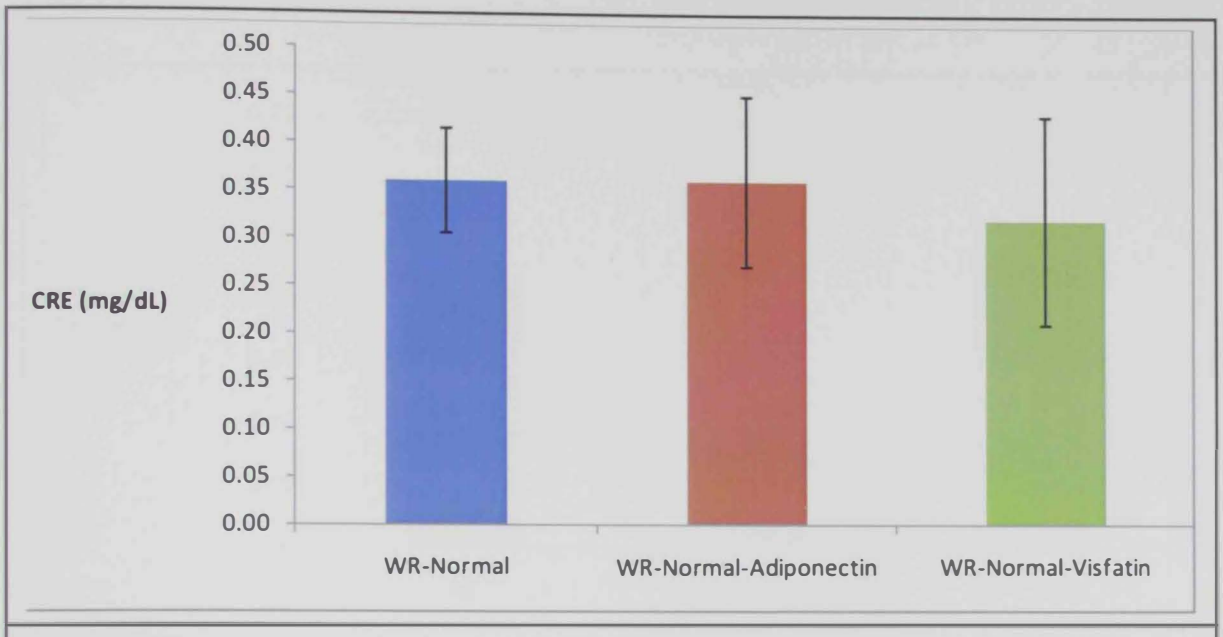



Figure (125) shows creatinine plasma level in normal Wistar rats treated with either adiponectin or visfatin. No difference was observed in the three groups.

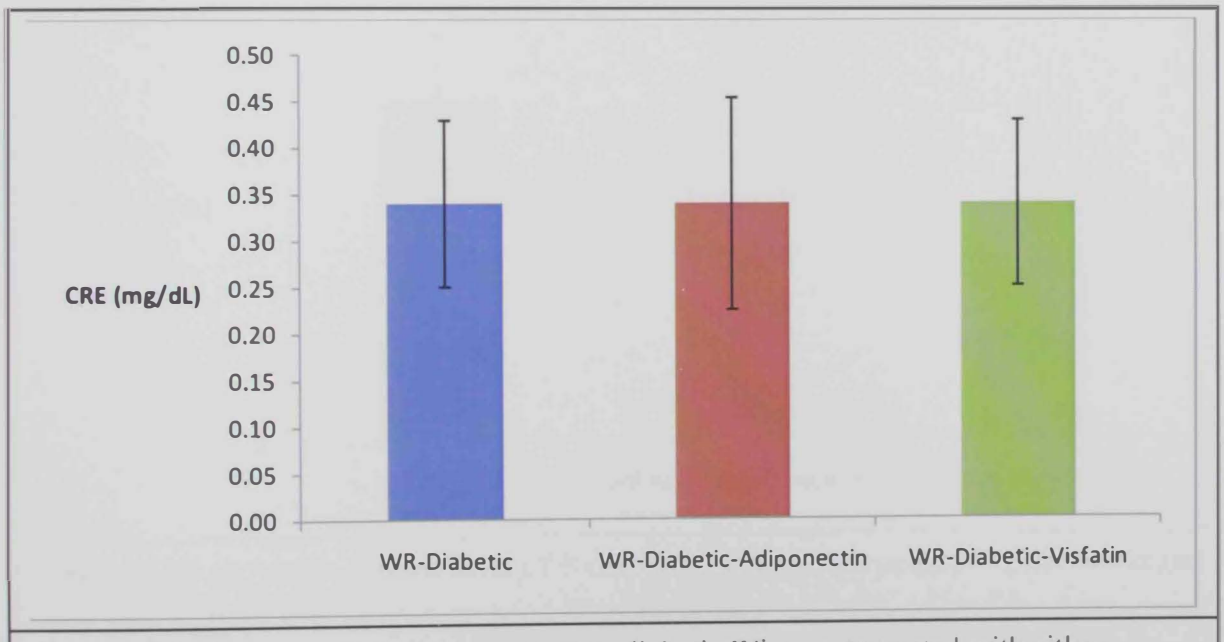



Figure (126) shows creatinine plasma level in diabetic Wistar rats treated with either adiponectin or visfatin. No difference was observed in the three groups.

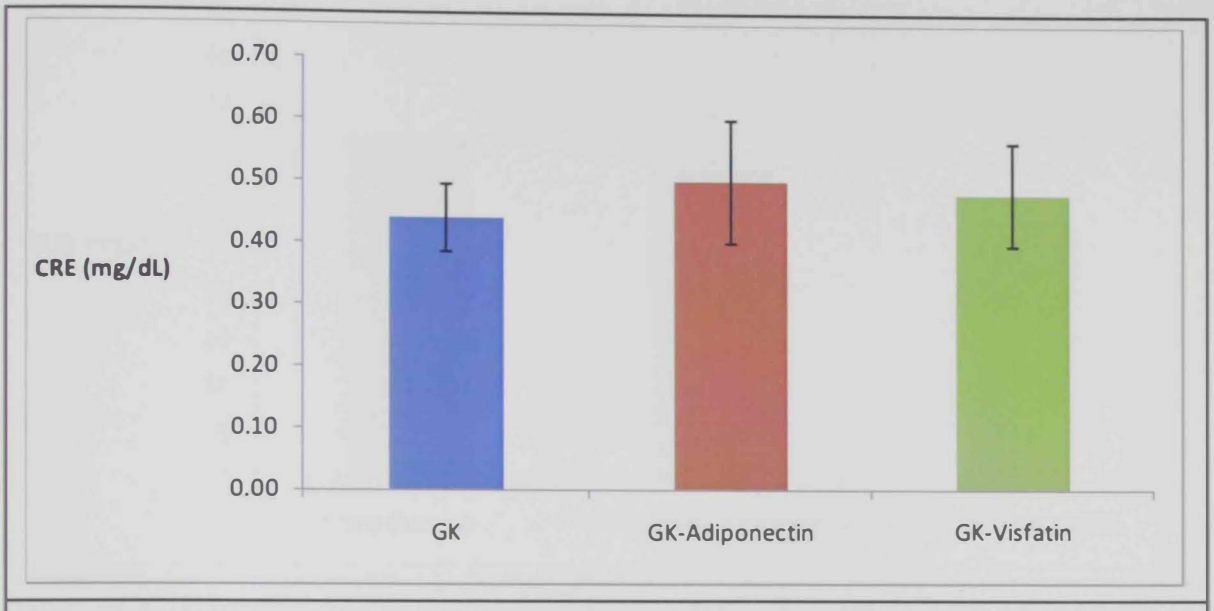



Figure (127) shows creatinine plasma level in GK rats treated with either adiponectin or visfatin. No difference was observed in the three groups.

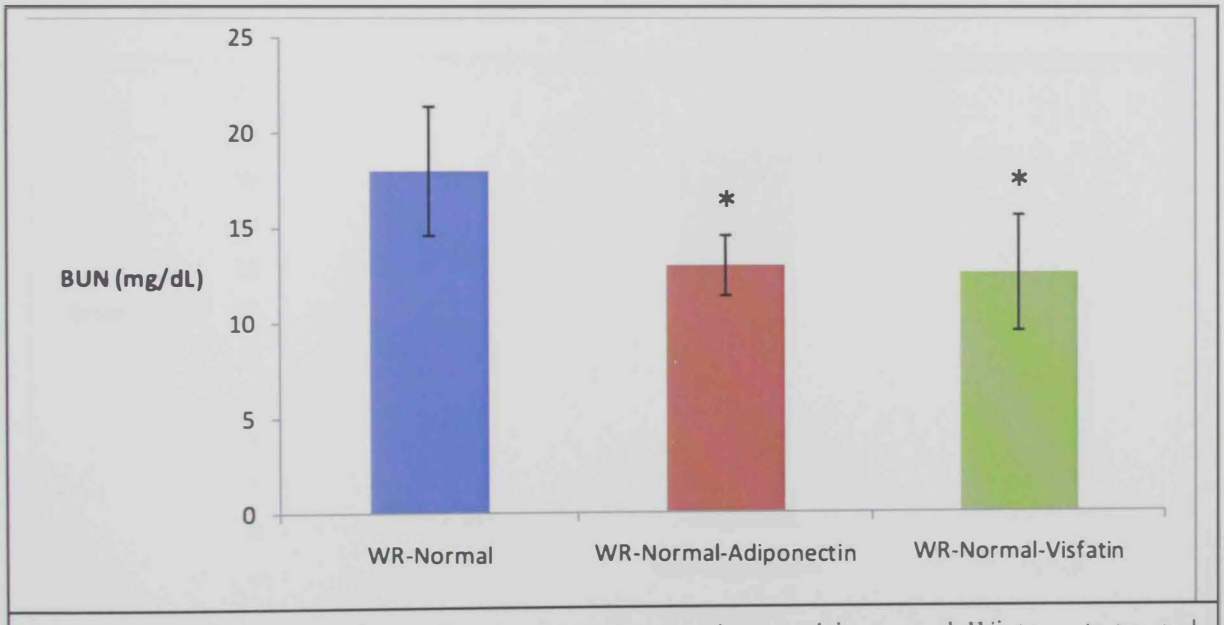



Figure (128) shows the plasma level of Blood urea nitrogen I in normal Wistar rats treated with either adiponectin or visfatin. Note that adiponectin- and visfatin-treated rats displayed significant ($p < 0.05$) reduction in plasma BUN level.

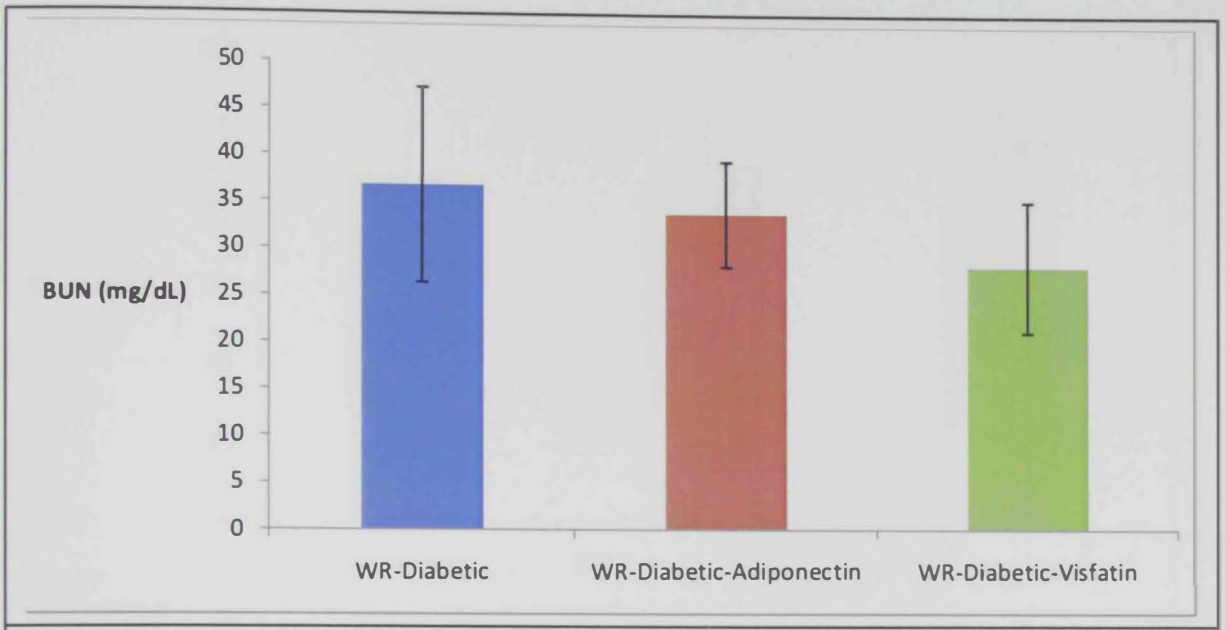



Figure (129) shows the plasma level of blood urea nitrogen in normal Wistar rats treated with either adiponectin or visfatin. No significant difference between the three groups.

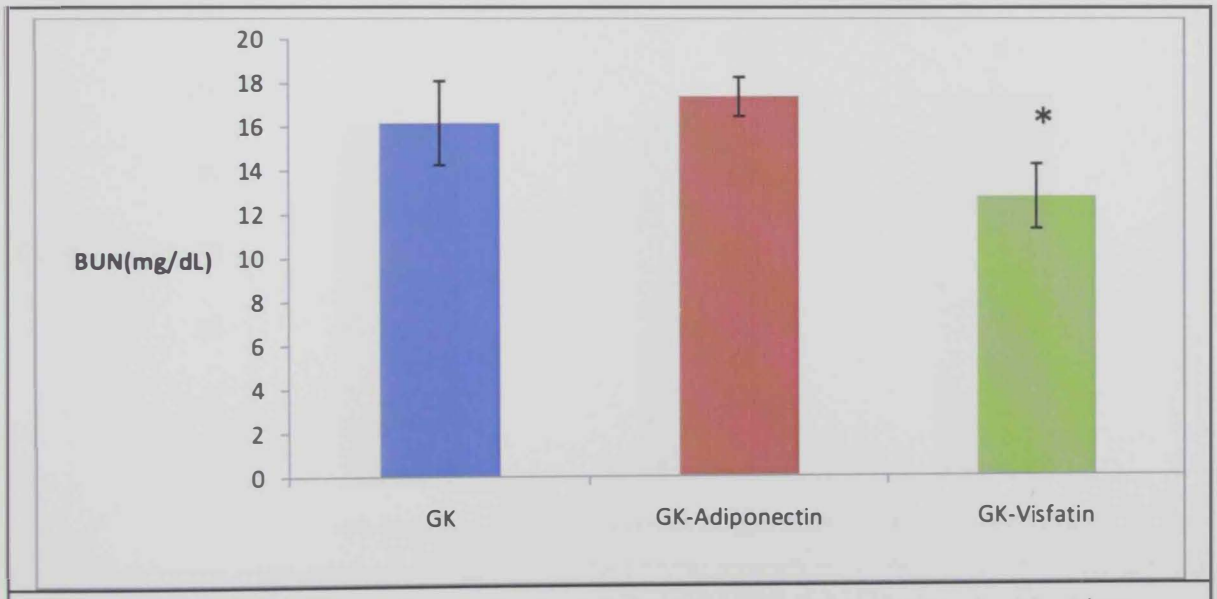



Figure (130) shows the blood urea nitrogen plasma level in GK rats treated with either adiponectin or visfatin. Note that Visfatin-treated rats displayed significant ($p < 0.05$) reduction in plasma BUN level.

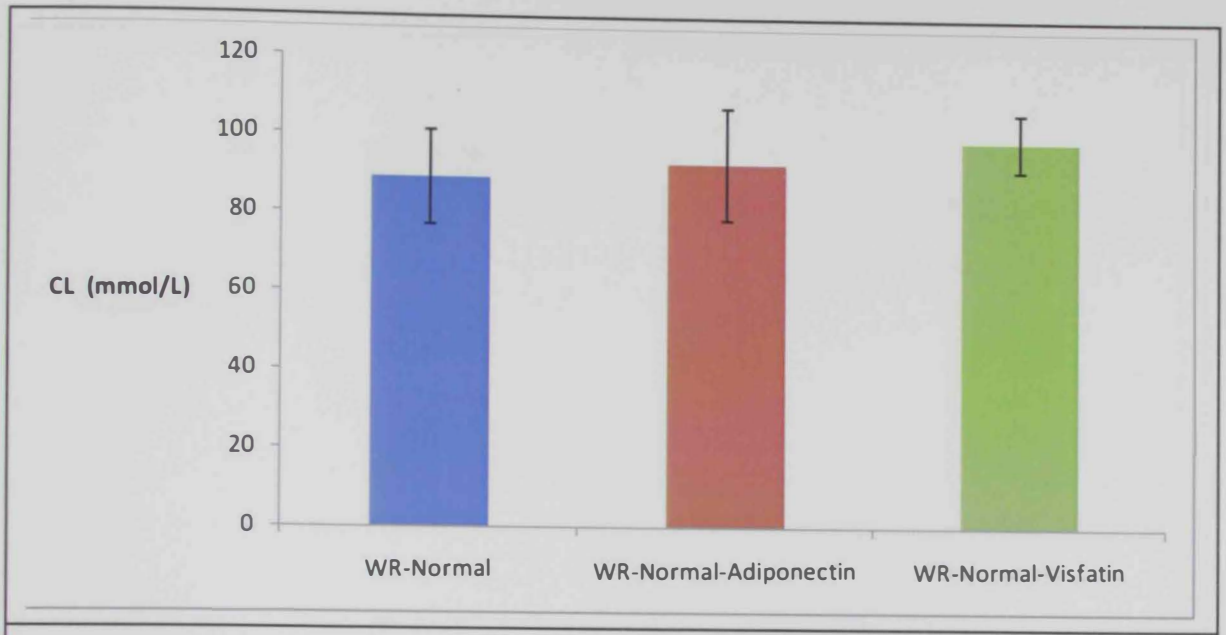



Figure (131) shows plasma chloride plasma level in normal Wistar rats treated with either adiponectin or visfatin. No difference was observed in the three groups.

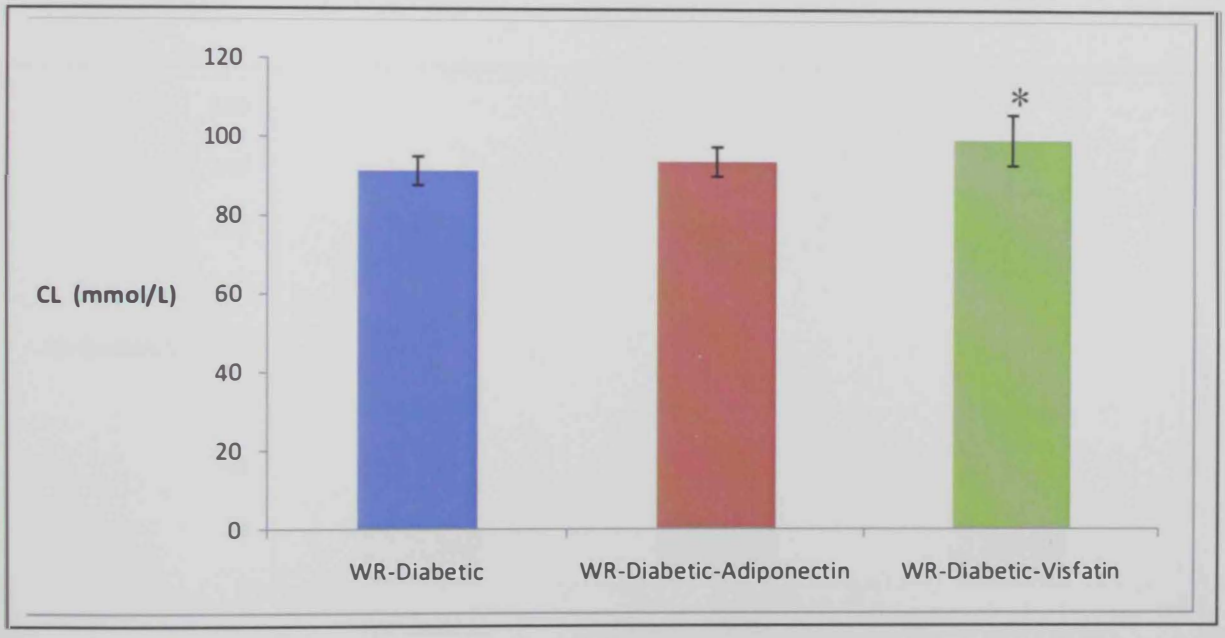



Figure (132) shows plasma levels of chloride in diabetic Wistar rats treated with either adiponectin or visfatin. Note that Wistar diabetic rats treated with visfatin show significant ($p < 0.05$) increase on Chloride plasma level.

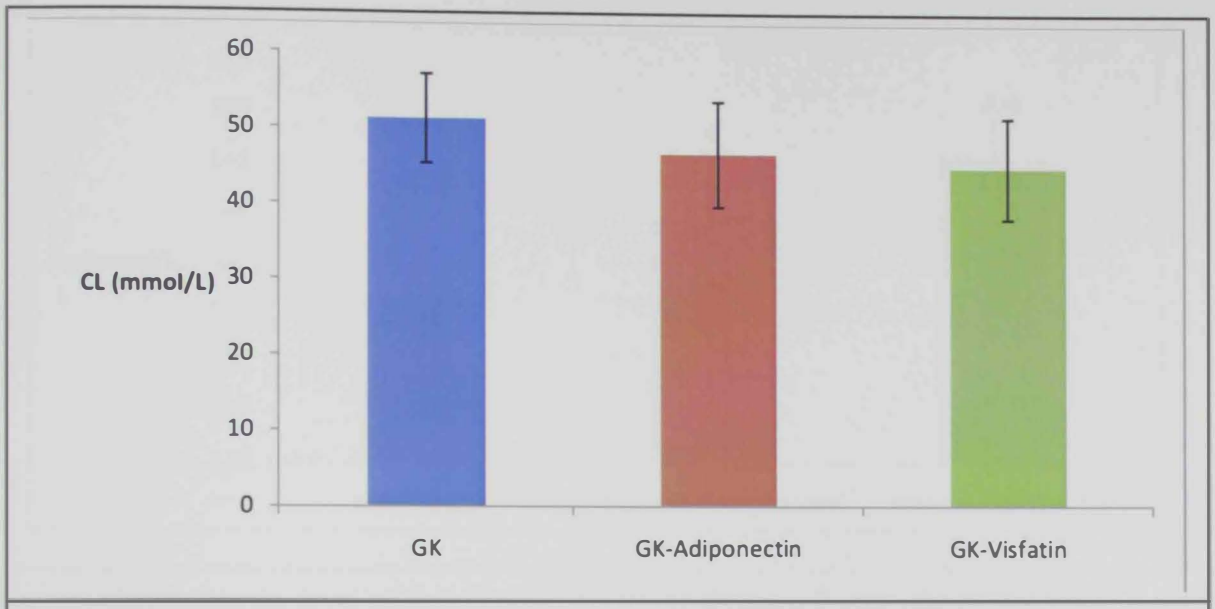



Figure (133) shows plasma level of chloride in GK rats treated with either adiponectin or visfatin. No difference was observed in the three groups.

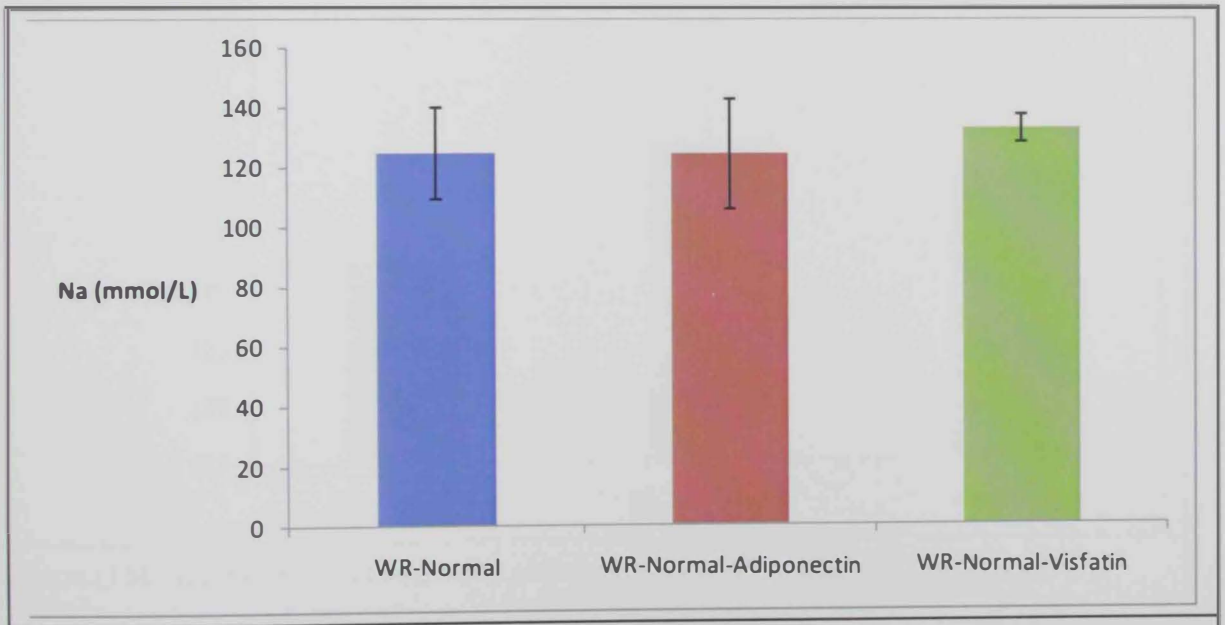



Figure (134) shows the level of plasma sodium (Na) in normal Wistar rats treated with either adiponectin or visfatin. There was no significant difference between the 3 groups.

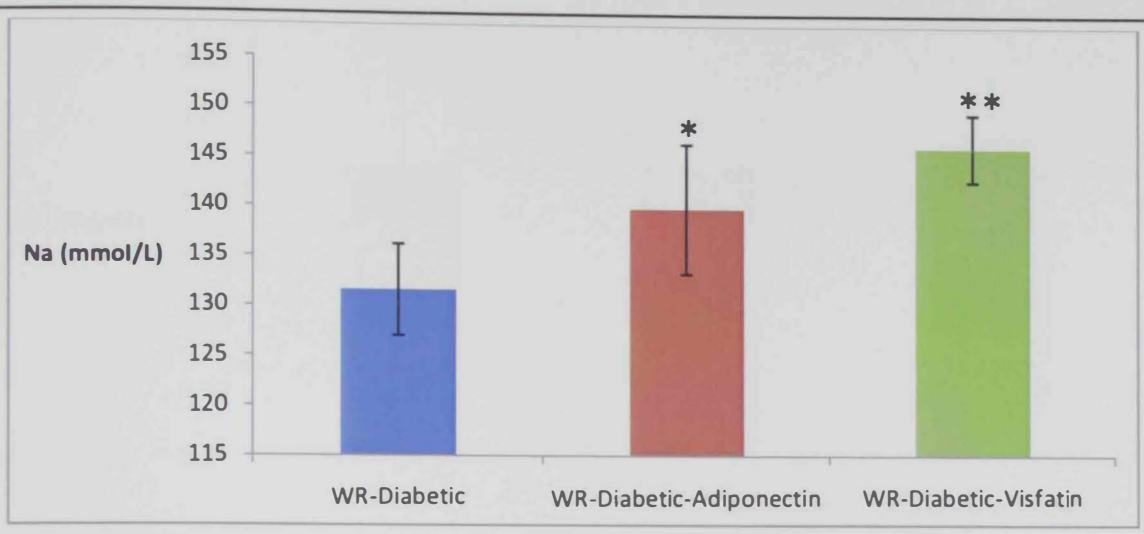



Figure (135) shows the level of plasma sodium (Na) in diabetic Wistar rats treated with either adiponectin or visfatin. Note that Wistar Diabetic rats treated with Adiponectin or Visfatin show significant ($p < 0.05$) increase on sodium plasma level

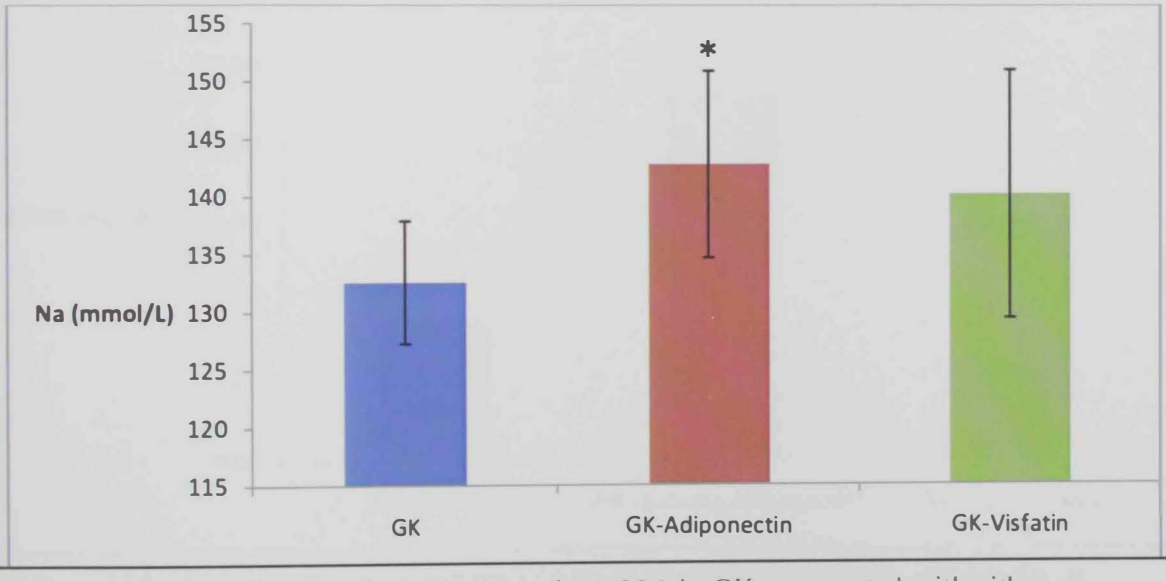



Figure (136) shows the level of plasma sodium (Na) in GK rats treated with either adiponectin or visfatin. Note that GK rats treated with adiponectin show significant ($p < 0.05$) increase on plasma sodium leve.l

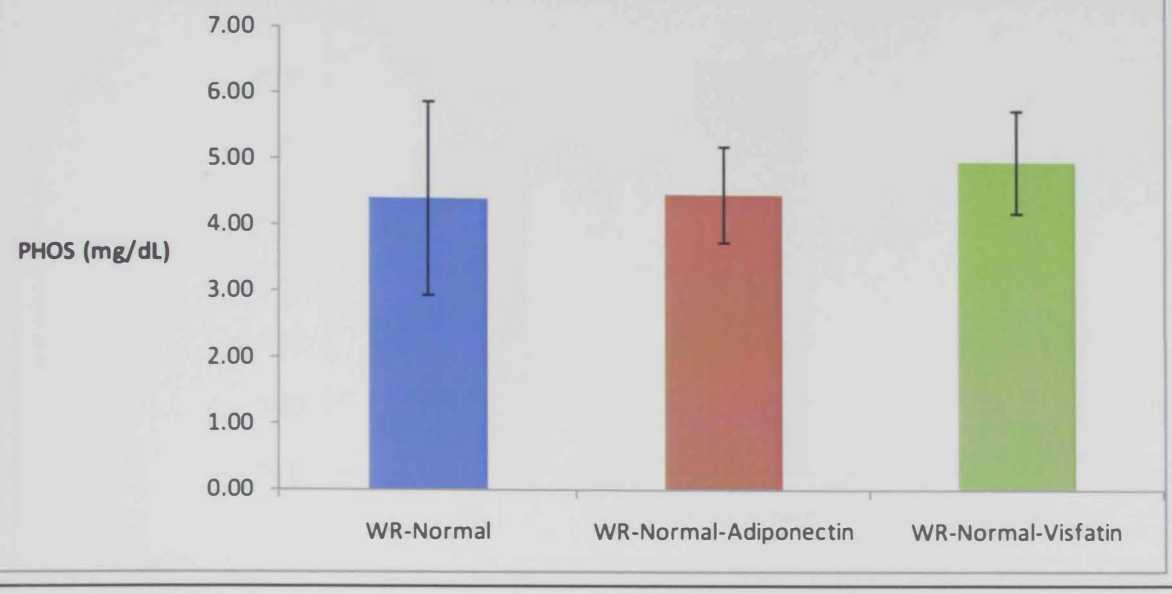



Figure (137) shows the effect of treatment of normal Wistar rats with adiponectin or visfatin on plasma phosphorus level. There is no significant difference between the 3 groups.

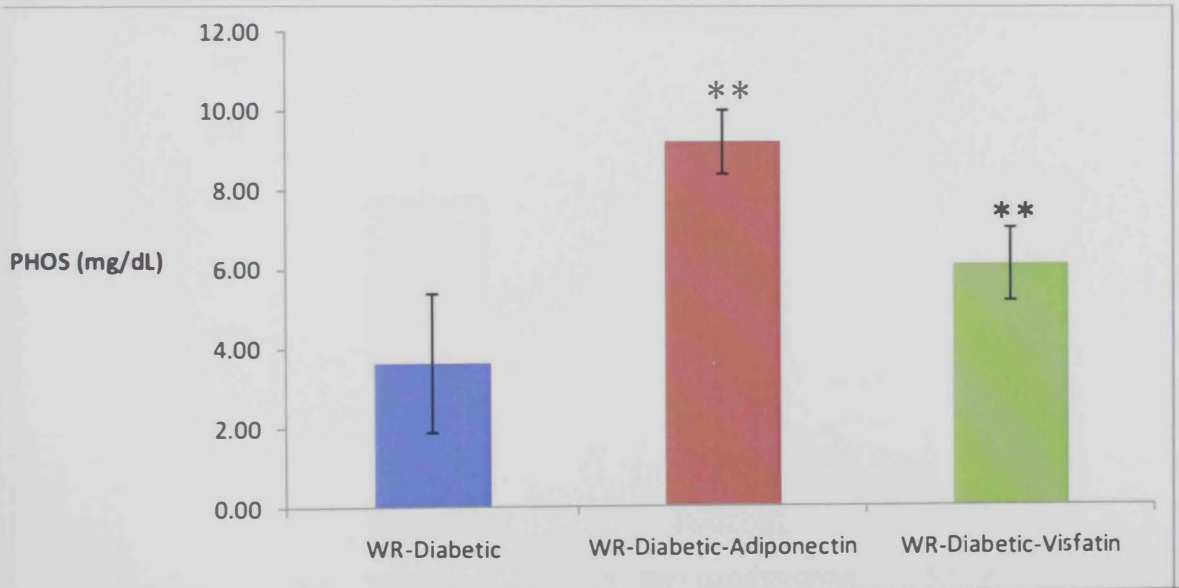



Figure (138) shows the effect of treatment of diabetic Wistar rats with adiponectin or visfatin on plasma phosphorus level. Note that Wistar diabetic rats treated with either adiponectin or visfatin show significant ($p < 0.005$) increase in plasma phosphorus level.

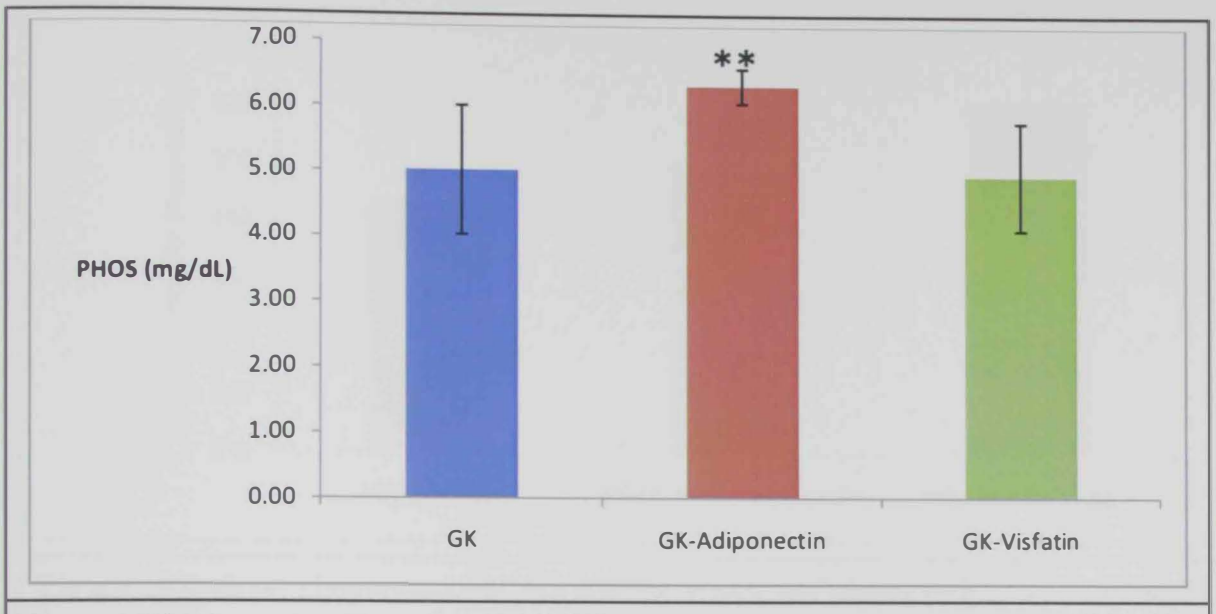



Figure (139) shows the effect of treatment of GK rats with adiponectin or visfatin on plasma phosphorus level. Note that GK rats treated with adiponectin displayed significant ($p < 0.005$) increase in plasma phosphorus level.

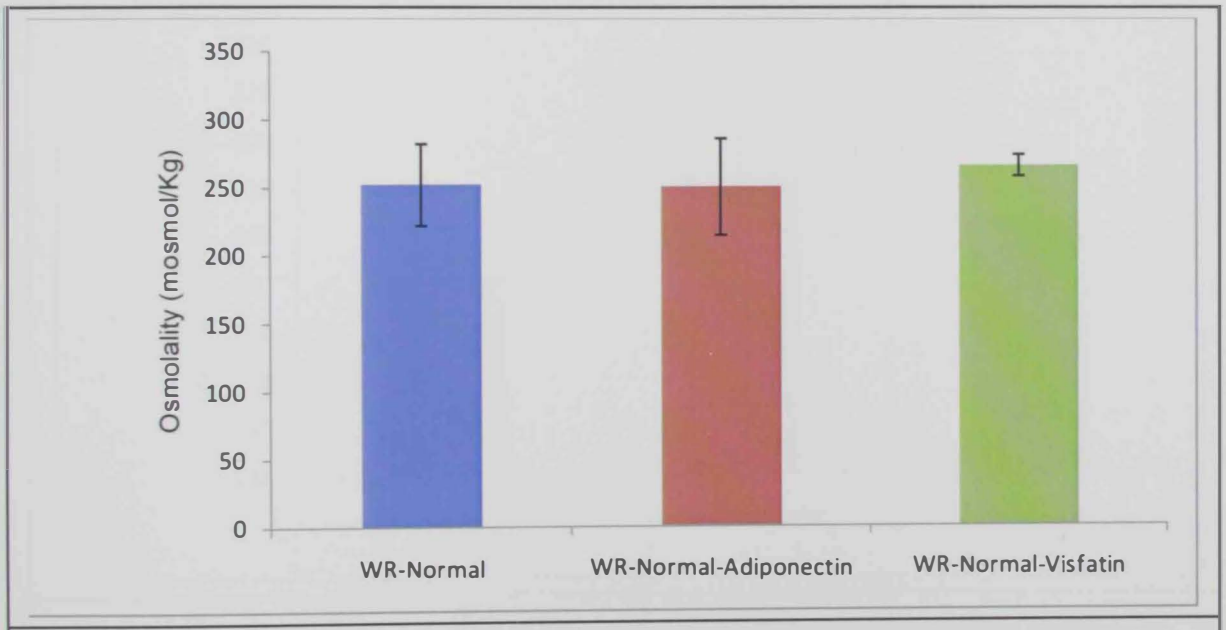



Figure (140) shows plasma osmolality in normal Wistar rats treated with either adiponectin or visfatin. There was no significant difference between the 3 groups.

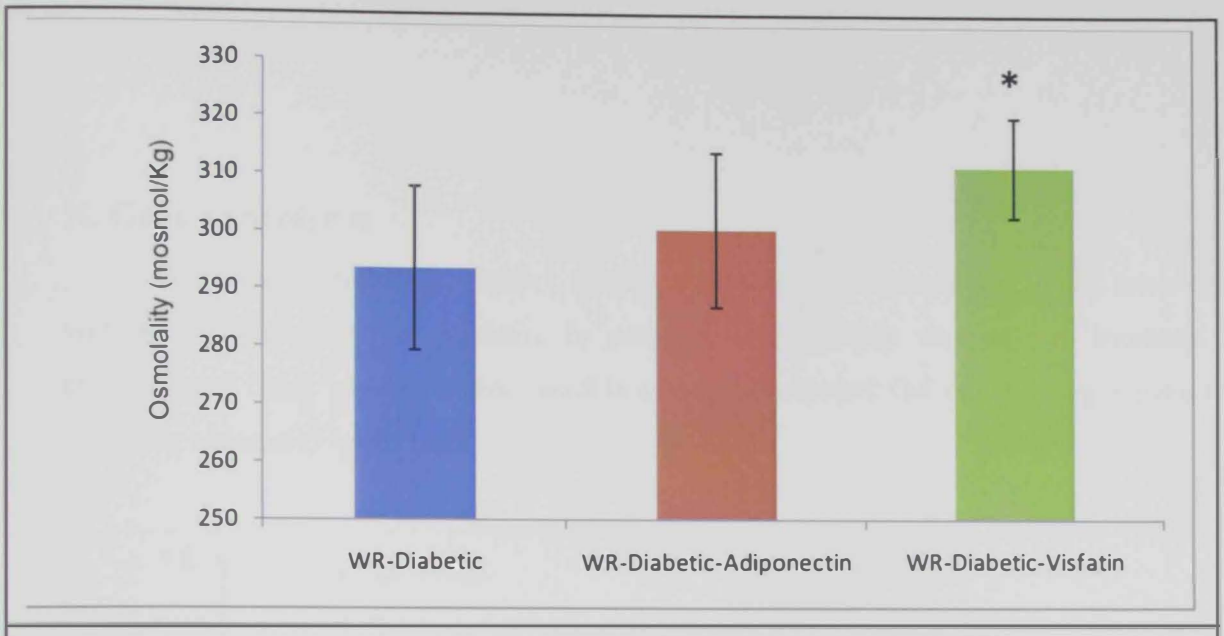



Figure (141) shows plasma osmolality in diabetic Wistar rats treated with either adiponectin or visfatin. Note that Wistar Diabetic rats treated with visfatin show significant ($p< 0.05$) increase on osmolality

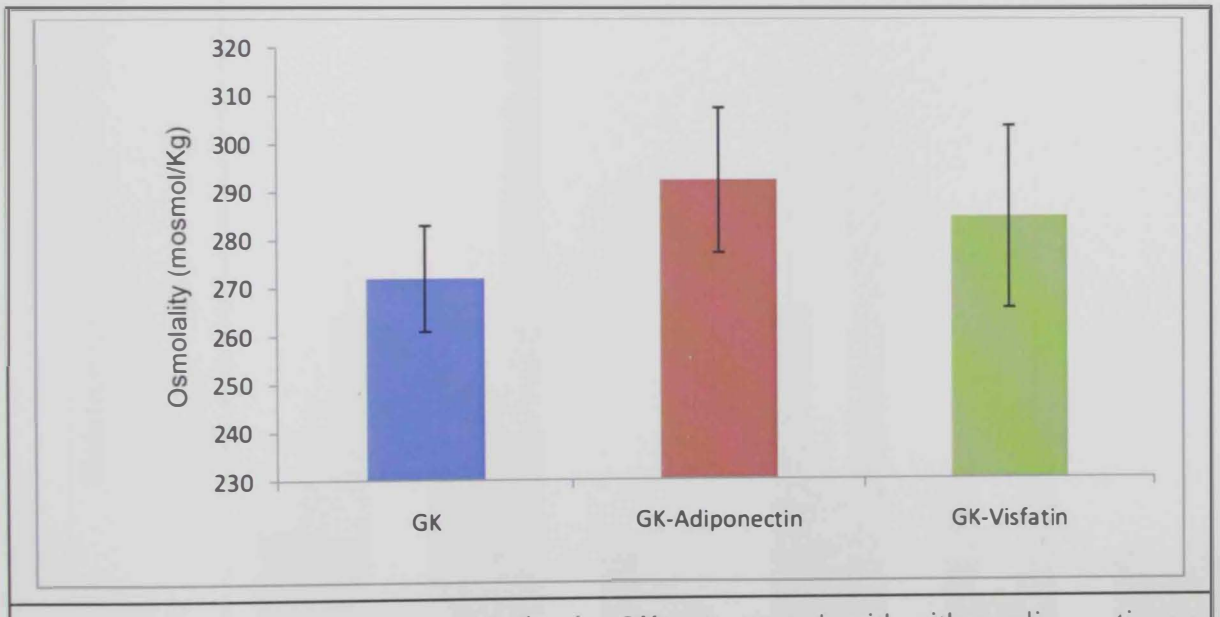



Figure (142) shows plasma osmolality in GK rats treated with either adiponectin or visfatin. GK rats treated with adiponectin show significant ($p< 0.05$) increase on osmolality

## X. Gene expression

Many genes including, Hmox1, Hspa4, and Pdx1 were upregulated in GK rats treated with both adiponectin and visfatin. In contrast, only visfatin was able to increase the expression of Gpx2 which was decreased in adiponectin-treated GK rats. Glucagon gene was markedly reduced (Figure 143).

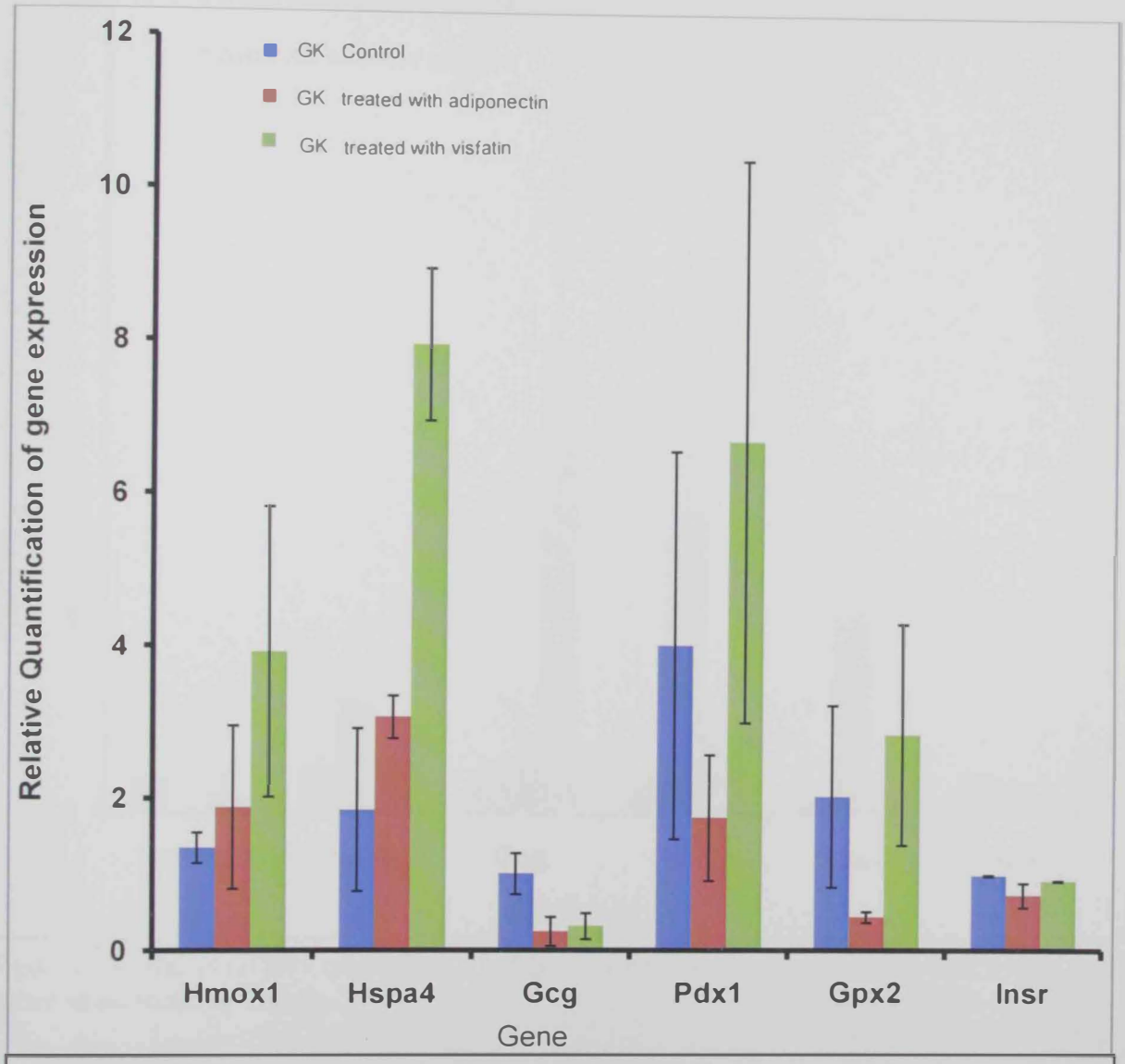



Figure (143) shows relative quantification of gene expression for GK rats treated with either adiponectin or visfatin

The expression of Hspa4, Gpx2 and Pdx1 was markedly elevated in normal Wistar rats treated with adiponectin. The expression of Pdx1 and Gcg was increased in normal Wistar rats treated with visfatin (Figure 144)

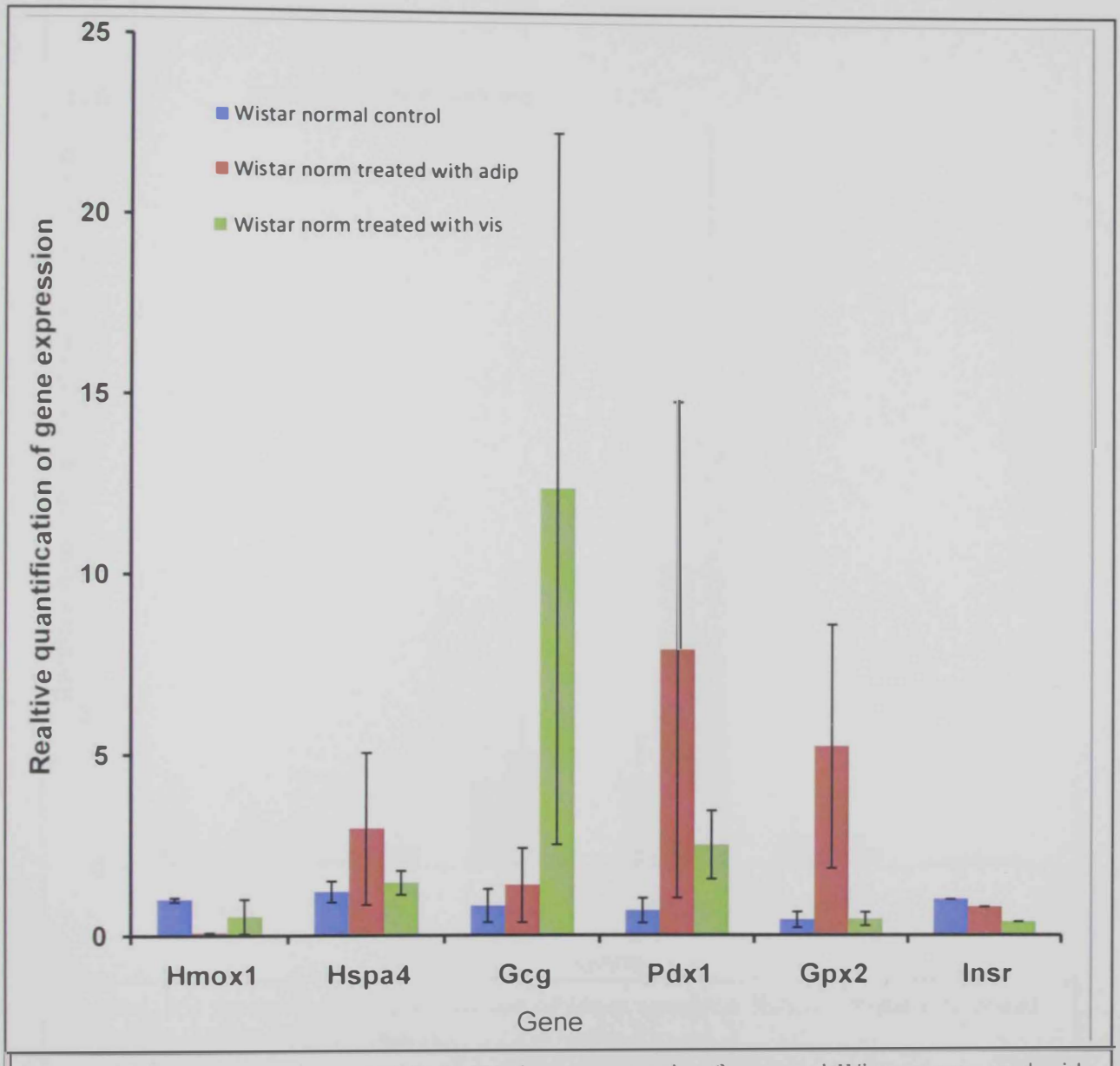



Figure (144) shows relative quantification of gene expression for normal Wistar rats treated with either adiponectin or visfatin.

Pdx1 gene increased in diabetic Wistar rats treated with either adiponectin or visfatin compared to control (Figure 145).

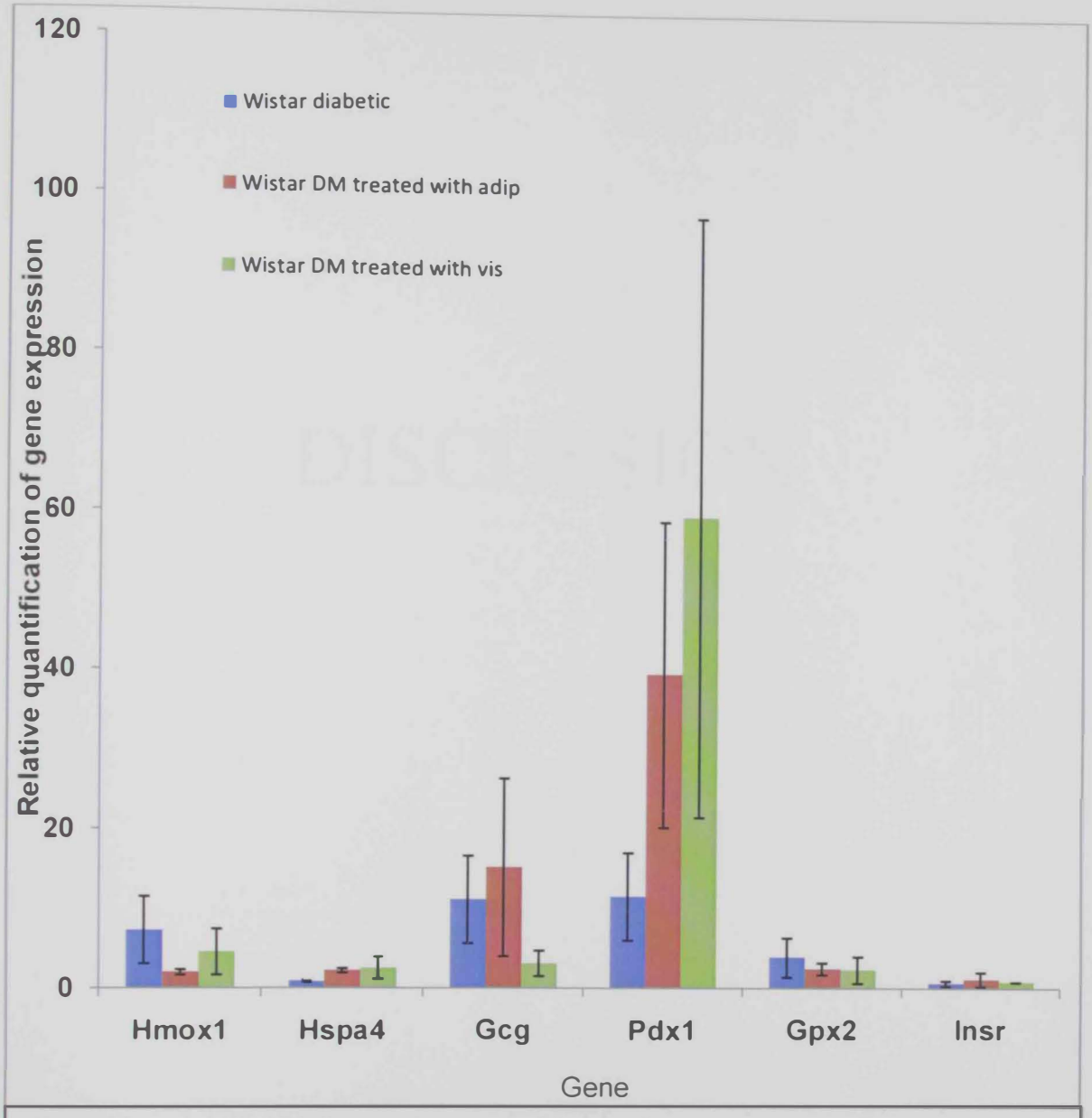



Figure (145) shows relative quantification of gene expression diabetic Wistar rats treated with either adiponectin or visfatin.

# DISCUSSION

# I. Effect of adiponectin and visfatin on metabolic parameters of animal models of diabetes

## I.1. Body weight

Body weight gain is an important factor in the management of diabetes. An ideal drug in the treatment of diabetes should decrease weight in obese patients and be neutral in lean patients. Neither normal, diabetic Wistar nor GK rats increased weight significantly after treatment with either adiponectin or visfatin. However, a steady body weight gain was observed in all animals investigated. It is not clear why these adipocytokines failed to improve weight. Our results did not corroborate those of (72) who reported that intracerebroventricular dose of adiponectin reduced body weight. They showed that the ability of adiponectin to increase weight was due to its ability to stimulate energy expenditure.

Although, the relationship of visfatin and body weight has been examined (73), the direct effect of this adipocytokine on body weight has not been investigated.

## I.2. Blood glucose

Treatment of diabetic Wistar rats with adiponectin and visfatin induced significant decrease in blood glucose level in week 4 of the experiment. A similar effect was observed in GK rats. However, in diabetic rats, the normalization of blood glucose level was followed by an increase from weeks 5- 8. The reason for this fluctuation is not clear. It may be due to overwhelming of pancreatic beta cell by free radicals observed in diabetes. The observation on the effect of adiponectin on glucose lowering effect corroborates those of others (65-66). The ability of adiponectin to improve blood glucose level may be due to the fact that adiponectin is capable of stimulating energy expenditure (72). With regards to visfatin, it has been reported that visfatin has an insulin-mimetic action (82).

### I.3. Glucose tolerance test

Visfatin and adiponectin-treated rats have better glucose handling 60 min after glucose challenge in normal Wistar and GK rats. No significant improvement in blood glucose level was observed after glucose challenge in diabetic Wistar rats. The lack of effect of adiponectin and visfatin in the diabetic Wistar rats may be due to deranged cellular and biochemical factors in diabetic rats. It has been shown for example that calcium is deranged in diabetes (67). Since calcium is a key factor in cellular transduction, the effect of these agents may fail to trigger the signal transduction pathway that results in insulin secretion from pancreatic better cells.

## II. Immunolocalization of adiponectin and visfatin in the pancreas

Adiponectin and visfatin were both located to the islet of Langerhans in Wistar as well as GK rats. Treatment of the animal models improves islet cell morphology and size. This shows that adiponectin may have tissue regenerative properties capable of inducing a larger and more functional pancreatic beta cell mass. The large number of adiponectin and visfatin positive cells implies a functional role in the islet of Langerhans. Their presence in the endocrine pancreas also indicated that they may influence the functions of other bioactive agents in the islets of Langerhans.

## III. Immunolocalization of adiponectin and visfatin with pancreatic hormones in the pancreas

Adiponectin co-localizes with insulin in the endocrine pancreas of Wistar and GK rats. The number of adiponectin-positive cells decreased significantly after the onset of diabetes. This shows that adiponectin are indeed located in the pancreatic beta cell, which are damaged as a result of diabetes.

It was intriguing to know that some adiponectin-containing cells also have either insulin or somatostatin. The role of the dual co-localization is unknown. Although it has been shown that the number of adiponectin receptor is decreased in pancreas of rats (74), it is clear why adiponectin ligand decreased with the onset of type diabetes

Similarly, visfatin also co-localized with insulin in pancreatic islets of GK, normal and diabetic Wistar rats. This indicates that visfatin may play a role in the regulation of beta cell function. In fact a recent publication shows that visfatin can stimulate insulin release from clonal cell line (68). In addition to the presence of visfatin in pancreatic islet cells, visfatin was also observed in glucagon-secreting cells. In the same manner as for adiponectin, visfatin co-localized with PP in the pancreas of Wistar rats treated with visfatin.

All of these observations support a role for adiponectin and visfatin in the metabolism of pancreatic hormones at the level of the endocrine pancreas

## IV. Immunolocalization of adiponectin and visfatin in the fat tissue

In order to verify previous reports that adiponectin and visfatin are located to adipose tissue (82), we stained for these two adipocytokines in subcutaneous as well as visceral fat tissues. These two adipocytokines are well represented in both subcutaneous and visceral fats. However, visfatin appear to be more expressed in the epididymal fat of GK rat. A recent report (75) showed that visfatin expression is elevated in the omental fat of pregnant woman. This observation is similar to ours since the metabolism of glucose is deranged in the GK rat as well as in many pregnancy albeit physiologically.

## V. Effect of adiponectin and visfatin on pancreatic beta cell ultra-structure

Treatment of either normal or diabetic Wistar or GK rats with either adiponectin or visfatin improved pancreatic islet cell morphology, with increased number of secretory granules. The reason for this effect is not clear. It is however, possible that these adipocytokines may have proliferative effect on the endocrine pancreas. Some studies have confirmed the protective effect of adiponectin on pancreatic beta cell line (76).

## VI. Effect of adiponectin and visfatin on plasma insulin and glucagon level

The plasma levels of insulin decreased significantly in normal Wistar and GK rats treated with visfatin. In contrast, the plasma level of insulin increased dramatically in diabetic Wistar rats treated with visfatin. The cause of the difference is not clear. However, it is possible that visfatin is more effective when the need for insulin is more severe. In contrast, the plasma level of diabetic Wistar and GK rats treated with adiponectin increased significantly at the end of the experiment. The data obtained on insulin secretion agrees with that obtained on glucagon, because the plasma level of glucagon changed concurrently with that of plasma insulin. This observation corroborates that of (77), who showed that adiponectin can stimulate in vivo as well as in vitro insulin release in normal mice. In addition, visfatin has also been shown to stimulate insulin release from pancreatic cell line (68). The reason for the reduction in the level of insulin released after treatment of normal Wistar and GK rats with visfatin is not clear.

## VII. In vitro effect of adiponectin and visfatin on insulin and glucagon release from the pancreas

Adiponectin inhibited insulin release from Wistar rats. In contrast, visfatin has no significant effect on insulin release from the in vitro pancreas. The addition of cholinergic and adrenergic antagonists to adiponectin increased insulin release.

In contrast, the addition of atropine and yohimbine inhibited visfatin-induced response in GK rats. This clearly shows that adiponectin and visfatin act via different signaling pathways.

Adiponectin has no effect on glucagon secretion from the pancreas of GK rats but inhibited glucagon release from the pancreas of normal Wistar rats. Visfatin also inhibit glucagon release at one concentration but increased glucagon release at the other. The reason for this phenomenon is not clear. It is possible that action of visfatin is biphasic.

The present observation contradicts those of Okamoto et al 2008, and Brown et al, 2009 who reported that adiponectin and visfatin can stimulate insulin release respectively.

## VIII. Effect of adiponectin and visfatinon biochemical parameters

### VIII.1. Liver function tests

Visfatin cause significant increases in the albumin level of diabetic Wistar rats compared to adiponectin-treated and control Wistar rats. In contrast, adiponectin induced large increase in plasma albumin level in GK rats compared to control. The two adipocytokines appear to facilitate albumin production in diabetic models.

The level of GGT was not altered in GK and normal Wistar rats treated with either adiponectin or visfatin. Paradoxically, the plasma level GGT of diabetic Wistar rats treated adiponectin increased significantly. The reason for this is not clear. However, visfatin was able to significantly reduce LDH level in diabetic Wistar and GK rats. It has been shown that adiponectin can attenuate liver inflammation (70). This might imply that adiponectin may protect the liver from other noxious factors. In addition the two adipocytokines were able to reduce plasma ALP and AST level in normal Wistar and GK rats.

### VIII.2. Lipids

It was of interest to note that visfatin increased the plasma level of triglycerides in normal Wistar rats. Adiponectin did not significantly affect triglyceride level in normal Wistar rats. In addition, total cholesterol increased in normal Wistar rats treated with visfatin and in diabetic Wistar treated with either adiponectin or visfatin. The cause of these discrepancies is unknown.

### VIII.3. Proteins

Total protein was not modified by the administration of either adiponectin or visfatin except for GK rats treated with adiponectin. This effect supports the protective role of adiponectin in the liver (70).

### VIII.4. Kidney function tests and electrolytes

Although the level of creatinine was not altered by adipocytokine treatment, the plasma level of blood urea nitrogen was significantly reduced in normal Wistar rats treated with either adiponectin or visfatin and in GK rats treated with visfatin. Many studies have shown that the level of adiponectin is high in patients with renal failure (71). However, it has been reported that very low instead of high plasma adiponectin level is recognized as a risk factor of cardiovascular morbidity and mortality in patients suffering from chronic kidney failure (78).

Plasma sodium was significantly increased in diabetic Wistar rats treated with either adiponectin or visfatin and in GK rats treated with adiponectin but was not altered in normal Wistar rats. The pattern of plasma level of chloride was similar to that sodium. The level of chloride rose in tandem with that of sodium in diabetic Wistar rats treated with visfatin.

The increase in plasma level of sodium may be due to the enhancement of insulin because insulin has been shown to cause sodium retention. The plasma level of phosphorus rose in diabetic Wistar rats treated with either adiponectin or visfatin and in GK rats treated with adiponectin. This may be due to a general improvement of the metabolic parameters.

## IX. Gene expression

Adiponectin and visfatin increased genes that are to protect pancreatic islet cells. These adipocytokines may be exerting their effects via these genes. The administration of adiponectin as well as visfatin induced pdx1 in both normal and diabetic Wistar rats. The ability of these adipocytokines to stimulate pdx1 is intriguing because, pdx1 has been shown to be involved in the development of the pancreas (79). The importance of ddx1 in pancreatic development was shown when transgenic mice became completely apancreatic after tetracycline injection the course of pregnancy (80). It does appears that adipocytokines such as visfatin can help in the maintenance of pancreatic islet integrity via stimulation of genes involved in the development and maturation of pancreatic beta cell.

## Conclusion

Adiponectin and visfatin improved glucose tolerance in Wistar as well as GK rats. In addition, the number and quality of pancreatic beta cells improved after treatment with adiponectin and visfatin leading to increase in the plasma level of insulin. Liver and kidney parameters including lactic acid dehydrogenase, alkaline phosphatase, blood urea nitrogen decreased significantly after treatment with either adiponectin or visfatin. In conclusion Adiponectin and visfatin ameliorate several metabolic parameters in animal models of type 1 and type 2 diabetes, resulting in improved glycemic control.

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# الملخص العربي


خلفية: داء السكري هو واحد من اضرابات الغددالأكثر شيوعا والتي تصيب أكثر من 6 % من سكان العالم فالحالات المسجلة لهذا المرض المزمن في تزايد مستمر في أجزاء كثيرة من العالم بما في ذلك الشرق الأوسط.
يحدث داء السكري بسبب خلل في إنتاج الإنسولين أوخلل في عمله وقد لوحظ الخلل في إنتاج الإنسولين في مرضى السكرى النوع الأول بينما كان الخلل في عمل الانسولين (مقاومة الإنسولين ) هو الغالب في مرضى السكري النوع الثاني والذي يصيب أكثر من 94 % من مرضى السكري

المواد وطريقة البحث : جرذان قسمت الي تسع مجموعات كل مجموعة مكونة من 12 جرذ من نوعين نوع يمثل مرضى السكري نوع الثاني Goto kakizaki في عمر شهرين و ثلاثة أشهر ونوع Wistar تم حقنه بمادة streptozotocin لتحفيز السكري من النوع الأول ، بعد اسبوع من الحقن وتأكد اصابته بالنوع الأول من السكري تم معالجته مع النوع الثاني بمادة visfatin ومادة adiponectin بمعدل جرعة ثابته 10 نانوجرام لكل كيلوجرام على مدار 14 أسبوع ، بعدها تم جمع عينات الدم من اجل اجراء تحاليل الكيمياء الحيوية مثل تحليل وظائف الكبد والكلى ، ومن ثم تم إزالة البنكرياس وتجزئته لعينات من أجل دراسات المجهر.

النتائج : إن مادتي adiponectin و visfatin حسنت مقاومة الجلوكوز في الجرذان المعالجة من النوعين بالإضافة إلى ذلك فإن أعداد وجودة خلايا بيتا البنكرياسية تحسنت بعد العلاج مما أدى لإرتفاع نسبة الإنسولين في بلازما الدم . كما تناقصت بعد العلاج بشكل واضح مؤشرات وظائف الكبد والكلى بما فيها
lactic acid dehydrogenase, alkaline phosphatase, blood urea nitrogen

الخلاصة : إن مادتي adiponectin و visfatin حسنت عوامل التمثيل الأيضي في النمط الحيواني المصاب بالنوع الأول أو الثاني من السكر و ذلك أدى لتحسن مراقبة ارتفاع السكر في الدم .

جامعة الإمارات العربية المتحدة
عمادة الدراسات العليا
برنامج ماجستير علوم البيئة

# تأثير الفيزفاتين والأديبونكتين على المؤشرات الأيضية لنمط حيواني مصاب بداء السكري من النوع الثاني

رسالة مقدمة من الطالبة /

ثريا سعيد علي الشامسي

مقدمة إلى /
جامعة الإمارات العربية المتحدة
استكمالاً لمتطلبات الحصول على درجة الماجستير في علوم البيئة

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